

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 15 November 2000 (15.11.00)	
International application No. PCT/GB00/01035	Applicant's or agent's file reference JPD/P10281PCT
International filing date (day/month/year) 20 March 2000 (20.03.00)	Priority date (day/month/year) 18 March 1999 (18.03.99)
Applicant NAPIER, Johnathan, A.	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

18 October 2000 (18.10.00)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Zakaria EL KHODARY Telephone No.: (41-22) 338.83.38
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PARENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

HALE, Stephen, Geoffrey
J.Y. & G.W. Johnson
Kingsbourne House
229-231 High Holbourn
London WC1V 7DP
ROYAUME-UNI

Date of mailing (day/month/year) 26 June 2001 (26.06.01)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference JPD/P10281PCT	
International application No. PCT/GB00/01035	International filing date (day/month/year) 20 March 2000 (20.03.00)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address

DEAN, John, Paul
Withers & Rogers
Goldings House
2 Hays Lane
London SE1 2HW
United Kingdom

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒ the person ☒ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

HALE, Stephen, Geoffrey
J.Y. & G.W. Johnson
Kingsbourne House
229-231 High Holbourn
London WC1V 7DP
United Kingdom

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input checked="" type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

I. Britel

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference JPD/P10281PCT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 00/ 01035	International filing date (day/month/year) 20/03/2000	(Earliest) Priority Date (day/month/year) 18/03/1999
Applicant THE UNIVERSITY OF BRISTOL		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

POLYSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☒ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1
☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/01035

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 38-40 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01035

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/54 C12N9/10 C12N1/21 A01H5/00 C07C57/03
C07C57/12 A61K38/45 A61K31/202 A23L1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE SWALL 'Online! EMBL Heidelberg, Germany; ID: YLF4_CAEEL, AC: Q03574, 1 February 1994 (1994-02-01) WILSON R ET AL.: "2.2 Mb of contiguous nucleotide sequence from chromosome III of C. elegans" XP002143744 abstract</p> <p style="text-align: center;">--- -/--</p>	1-12, 18, 21, 24

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

28 July 2000

Date of mailing of the international search report

17.08.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Oderwald, H

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01035

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMINV 'Online! EMBL Heidelberg, Germany; ID: CEC40H1, AC: Z19154, 27 December 1992 (1992-12-27) BERKS M: "Caenorhabditis elegans cosmid C40H1" XP002143745 see nucleotides 18500 to 20600 abstract</p>	16,17
X	<p>--- JAMES D W ET AL: "DIRECTED TAGGING OF THE ARABIDOPSIS FATTY ACID ELONGATION1 (FAE1) GENE WITH THE MAIZE TRANSPOREN ACTIVATOR" PLANT CELL,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 7, March 1995 (1995-03), pages 309-319, XP002911493 ISSN: 1040-4651 cited in the application the whole document</p>	1-3,7,8, 16-18, 21-23, 27-29
A	<p>--- WATTS J L AND BROWNE J: "Isolation and characterization of a delta5-fatty acid desaturase from Caenorhabditis elegans" ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 362, no. 1, 1 February 1999 (1999-02-01), pages 175-182, XP002143742 the whole document</p>	22
A	<p>--- NAPIER ET AL: "Identification of a Caenorhabditis elegans delta6-fatty-acid-desaturase by heterologous expression in Saccharomyces cerevisiae" BIOCHEMICAL JOURNAL,GB,PORTLAND PRESS, LONDON, vol. 330, no. 2, March 1998 (1998-03), pages 611-614-614, XP002099453 ISSN: 0264-6021 the whole document</p>	23
A	<p>--- SALEM N ET AL: "Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 93, no. 93, January 1996 (1996-01), pages 49-54-54, XP002131822 ISSN: 0027-8424 the whole document</p> <p>--- -/--</p>	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01035

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OH ET AL: "EL02 and EL03, homologs of the Saccharomyces cerevisiae EL01 gene, function in fatty acid elongation and are required for sphingolipid formation" JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 28, 11 July 1997 (1997-07-11), pages 17376-17384, XP002119019 ISSN: 0021-9258 cited in the application the whole document	
P,X	WO 00 12720 A (ABBOTT LAB) 9 March 2000 (2000-03-09) the whole document	1-3, 7-31, 35

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/01035

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0012720 A	09-03-2000	AU 5696499 A	21-03-2000

To:
 WITHERS & ROGERS
 Attn. Dean, John Paul
 Goldings House
 2 Hays Lane
 London SE1 2HW
 UNITED KINGDOM

NOTIFICATION OF TRANSMITTAL OF
 THE INTERNATIONAL SEARCH REPORT
 OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing (day/month/year) 17/08/2000	
Applicant's or agent's file reference JPD/P10281PCT	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/GB 00/01035	International filing date (day/month/year) 20/03/2000
Applicant THE UNIVERSITY OF BRISTOL	

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland
 Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.


☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority  European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Mireille Claudepierre
--	---

These Notes are intended to give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/PEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference JPD/P10281PCT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 00/ 01035	International filing date (day/month/year) 20/03/2000	(Earliest) Priority Date (day/month/year) 18/03/1999
Applicant THE UNIVERSITY OF BRISTOL		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

POLYSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS

5. With regard to the **abstract**,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.

1



None of the figures.

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 38-40 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 7	C12N15/54 C07C57/12	C12N9 A61K3
	C12N1/21 A61K31/202	A01H5/00 A23L1/30
C07C57/03		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC 7 C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
EPO-Internal, WPI Data, PAJ, BIOSIS, STRAND		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE SWALL [Online] EMBL Heidelberg, Germany; ID: YLF4_CAEEL, AC: Q03574, 1 February 1994 (1994-02-01) WILSON R ET AL.: "2.2 Mb of contiguous nucleotide sequence from chromosome III of C. elegans" XP002143744 abstract</p> <p style="text-align: center;">--- -/-</p>	1-12, 18, 21, 24
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
<p>* Special categories of cited documents :</p> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>*Z* document member of the same patent family</p>		
Date of the actual completion of the international search		Date of mailing of the international search report
28 July 2000		17.08.00
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Oderwald, H

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, if appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMINV [Online] EMBL Heidelberg, Germany; ID: CEC40H1, AC: Z19154, 27 December 1992 (1992-12-27) BERKS M: "Caenorhabditis elegans cosmid C40H1" XP002143745 see nucleotides 18500 to 20600 abstract</p>	16,17
X	<p>--- JAMES D W ET AL: "DIRECTED TAGGING OF THE ARABIDOPSIS FATTY ACID ELONGATION1 (FAE1) GENE WITH THE MAIZE TRANSPOREN ACTIVATOR" PLANT CELL,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 7, March 1995 (1995-03), pages 309-319, XP002911493 ISSN: 1040-4651 cited in the application the whole document</p>	1-3,7,8, 16-18, 21-23, 27-29
A	<p>--- WATTS J L AND BROWNE J: "Isolation and characterization of a delta5-fatty acid desaturase from Caenorhabditis elegans" ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 362, no. 1, 1 February 1999 (1999-02-01), pages 175-182, XP002143742 the whole document</p>	22
A	<p>--- NAPIER ET AL: "Identification of a Caenorhabditis elegans delta6-fatty-acid-desaturase by heterologous expression in Saccharomyces cerevisiae" BIOCHEMICAL JOURNAL,GB,PORTLAND PRESS, LONDON, vol. 330, no. 2, March 1998 (1998-03), pages 611-614-614, XP002099453 ISSN: 0264-6021 the whole document</p>	23
A	<p>--- SALEM N ET AL: "Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 93, no. 93, January 1996 (1996-01), pages 49-54-54, XP002131822 ISSN: 0027-8424 the whole document</p> <p>--- -/--</p>	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication appropriate, of the relevant passages	Relevant to claim No.
A	OH ET AL: "ELO2 and ELO3, homologs of the Saccharomyces cerevisiae ELO1 gene, function in fatty acid elongation and are required for sphingolipid formation" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 28, 11 July 1997 (1997-07-11), pages 17376-17384, XP002119019 ISSN: 0021-9258 cited in the application the whole document ---	
P,X	WO 00 12720 A (ABBOTT LAB) 9 March 2000 (2000-03-09) the whole document -----	1-3, 7-31,35

Form PCT/ISA/210 (patent family annex) (July 1992)

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only	
PCT/GB-00/01035	
International Application No.	
20 MARCH 2000	20.03.2000
International Filing Date	
United Kingdom Patent Office PCT International Application	
Name of receiving Office and "PCT International Application"	
Applicant's or agent's file reference JPD/P10281PCT (if desired) (12 characters maximum)	

Box No. I	TITLE OF INVENTION		NOVEL POLYPEPTIDES	
Box No. II	APPLICANT			
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)			<input type="checkbox"/> This person is also inventor.	
THE UNIVERSITY OF BRISTOL SENATE HOUSE TYNDALL AVENUE BRISTOL BS8 1TH UNITED KINGDOM			Telephone No.	
			Facsimile No.	
			Teleprinter No.	
State (that is, country) of nationality:		GB [▲]	State (that is, country) of residence:	
		(UK)	GB [▲]	
This person is applicant for the purposes of:		<input type="checkbox"/> all designated States	<input checked="" type="checkbox"/> all designated States except the United States of America	<input type="checkbox"/> the United States of America only
			<input type="checkbox"/> the States indicated in the Supplemental Box	
Box No. III	FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)			
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)			This person is:	
NAPIER, JOHNATHAN A (C/O) IACR - LONG ASHTON RESEARCH STATION DEPARTMENT OF AGRICULTURE SCIENCES UNIVERSITY OF BRISTOL LONG ASHTON BRISTOL BS41 9AF UNITED KINGDOM			<input type="checkbox"/> applicant only <input checked="" type="checkbox"/> applicant and inventor <input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)	
State (that is, country) of nationality:		GB [▲]	State (that is, country) of residence:	
		(UK)	GB [▲]	
This person is applicant for the purposes of:		<input type="checkbox"/> all designated States	<input type="checkbox"/> all designated States except the United States of America	<input checked="" type="checkbox"/> the United States of America only
			<input type="checkbox"/> the States indicated in the Supplemental Box	
<input type="checkbox"/> Further applicants and/or (further) inventors are indicated on a continuation sheet.				
Box No. IV	AGENT OR COMMON REPRESENTATIVE: OR ADDRESS FOR CORRESPONDENCE			
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:			<input checked="" type="checkbox"/> agent <input type="checkbox"/> common representative	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)			Telephone No.	
DEAN, JOHN PAUL WITHERS & ROGERS GOLDINGS HOUSE 2 HAYS LANE LONDON SE1 2HW UNITED KINGDOM			+44 117 9253030	
			Facsimile No.	
			+44 117 9253530	
			Teleprinter No.	
<input type="checkbox"/> Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.				

Deleted

Added

Ro/GB

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

Regional Patent

- ☒ **AP ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | |
|--|--|
| <input checked="" type="checkbox"/> AE United Arab Emirates | <input checked="" type="checkbox"/> LR Liberia |
| <input checked="" type="checkbox"/> AL Albania | <input checked="" type="checkbox"/> LS Lesotho |
| <input checked="" type="checkbox"/> AM Armenia | <input checked="" type="checkbox"/> LT Lithuania |
| <input checked="" type="checkbox"/> AT Austria | <input checked="" type="checkbox"/> LU Luxembourg |
| <input checked="" type="checkbox"/> AU Australia | <input checked="" type="checkbox"/> LV Latvia |
| <input checked="" type="checkbox"/> AZ Azerbaijan | <input checked="" type="checkbox"/> MA Morocco |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina | <input checked="" type="checkbox"/> MD Republic of Moldova |
| <input checked="" type="checkbox"/> BB Barbados | <input checked="" type="checkbox"/> MG Madagascar |
| <input checked="" type="checkbox"/> BG Bulgaria | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia |
| <input checked="" type="checkbox"/> BR Brazil | |
| <input checked="" type="checkbox"/> BY Belarus | <input checked="" type="checkbox"/> MN Mongolia |
| <input checked="" type="checkbox"/> CA Canada | <input checked="" type="checkbox"/> MW Malawi |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein | <input checked="" type="checkbox"/> MX Mexico |
| <input checked="" type="checkbox"/> CN China | <input checked="" type="checkbox"/> NO Norway |
| <input checked="" type="checkbox"/> CR Costa Rica | <input checked="" type="checkbox"/> NZ New Zealand |
| <input checked="" type="checkbox"/> CU Cuba | <input checked="" type="checkbox"/> PL Poland |
| <input checked="" type="checkbox"/> CZ Czech Republic | <input checked="" type="checkbox"/> PT Portugal |
| <input checked="" type="checkbox"/> DE Germany | <input checked="" type="checkbox"/> RO Romania |
| <input checked="" type="checkbox"/> DK Denmark | <input checked="" type="checkbox"/> RU Russian Federation |
| <input checked="" type="checkbox"/> DM Dominica | <input checked="" type="checkbox"/> SD Sudan |
| <input checked="" type="checkbox"/> EE Estonia | <input checked="" type="checkbox"/> SE Sweden |
| <input checked="" type="checkbox"/> ES Spain | <input checked="" type="checkbox"/> SG Singapore |
| <input checked="" type="checkbox"/> FI Finland | <input checked="" type="checkbox"/> SI Slovenia |
| <input checked="" type="checkbox"/> GB United Kingdom | <input checked="" type="checkbox"/> SK Slovakia |
| <input checked="" type="checkbox"/> GD Grenada | <input checked="" type="checkbox"/> SL Sierra Leone |
| <input checked="" type="checkbox"/> GE Georgia | <input checked="" type="checkbox"/> TJ Tajikistan |
| <input checked="" type="checkbox"/> GH Ghana | <input checked="" type="checkbox"/> TM Turkmenistan |
| <input checked="" type="checkbox"/> GM Gambia | <input checked="" type="checkbox"/> TR Turkey |
| <input checked="" type="checkbox"/> HR Croatia | <input checked="" type="checkbox"/> TT Trinidad and Tobago |
| <input checked="" type="checkbox"/> HU Hungary | <input checked="" type="checkbox"/> TZ United Republic of Tanzania |
| <input checked="" type="checkbox"/> ID Indonesia | <input checked="" type="checkbox"/> UA Ukraine |
| <input checked="" type="checkbox"/> IL Israel | <input checked="" type="checkbox"/> UG Uganda |
| <input checked="" type="checkbox"/> IN India | <input checked="" type="checkbox"/> US United States of America |
| <input checked="" type="checkbox"/> IS Iceland | |
| <input checked="" type="checkbox"/> JP Japan | <input checked="" type="checkbox"/> UZ Uzbekistan |
| <input checked="" type="checkbox"/> KE Kenya | <input checked="" type="checkbox"/> VN Viet Nam |
| <input checked="" type="checkbox"/> KG Kyrgyzstan | <input checked="" type="checkbox"/> YU Yugoslavia |
| <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea | <input checked="" type="checkbox"/> ZA South Africa |
| | <input checked="" type="checkbox"/> ZW Zimbabwe |
| <input checked="" type="checkbox"/> KR Republic of Korea | Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet: |
| <input checked="" type="checkbox"/> KZ Kazakhstan | <input checked="" type="checkbox"/> AG Antigua and Barbuda |
| <input checked="" type="checkbox"/> LC Saint Lucia | <input checked="" type="checkbox"/> DZ Algeria |
| <input checked="" type="checkbox"/> LK Sri Lanka | |

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

- (i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available, in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below:
 - (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;
 - (iii) if, in Box No. II or in any of the sub-boxes of Box III, the inventor or the inventor/applicant is not inventor for the purposes of all designates States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or inventor(s) and next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;
 - (iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;
 - (v) if, in Box No. V, the name of any State (or OAPI) is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;
 - (vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. IV" and indicate for each additional earlier application the same type of information as required in Box No. VI;
 - (vii) if, in Box No. IV, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.
2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.
3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

Continuation of Box IV

D. G. Bannerman
N. M. Wilson
W. M. Blatchford
M. Adkins
A. J. Chettle
J. K. Hogg
J. P. Dean

I. S. Harrison
D. M. Pratt
B. J. N. Dempster
K. J. Barnfather
S. A. Beck
P. C. Turner
H. H. B. Wright

D. Croston
D. C. Jones
J. B. Jones

of

**WITHERS & ROGERS
GOLDINGS HOUSE
2 HAYS LANE
LONDON SE1 2HW
GB**

Box No. VI PRIORITY CLAIM

☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application: region Office	international application: receiving Office
item (1) (18.03.99) 18 MARCH 1999	990007.5 (990007.5)	GB [▲] [UK] ^{▲▲}		
item (2) (18.02.2000) [▲] 18 FEBRUARY 2000	0003869.5	GB [▲] [UK] ^{▲▲}		
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s): 1 & 2

* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA)
(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

Request to use results of earlier search: reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):

Date (day/month/year) Number Country (or regional Office)

ISA /

Box No. VIII CHECK LIST: LANGUAGE OF FILING

This international application contains the following number of sheets:

request : 4
description (excluding sequence listing part) : 27
claims : 4
abstract : 1
drawings : 4
sequence listing part of description : _____

Total number of sheets : 40

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney: reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other (specify):

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

DEAN, JOHN PAUL
WITHERS & ROGERS

For receiving Office use only

1. Date of actual receipt of the purported international application: 20 MARCH 2000 20.03.2000	2. Drawings: <input checked="" type="checkbox"/> received: <input type="checkbox"/> not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	
6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid.	

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EPO

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only		
Identification of IPEA		Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference JPD/SMH/P100281PCT
International application No. PCT/GB00/01035	International filing date (day/month/year) 20/03/2000	(Earliest) Priority date (day/month/year) 18/02/2000
Title of invention NOVEL POLYPEPTIDES		
Box No. II APPLICANT(S)		
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) THE UNIVERSITY OF BRISTOL Senate House Tyndall Avenue Bristol BS8 1TH United Kingdom		Telephone No.:
		Facsimile No.:
		Teleprinter No.:
State (that is, country) of nationality: GB		State (that is, country) of residence: GB
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) NAPIER, Bohnathan A IACR - Long Ashton Research Station Department of Agriculture Sciences University of Bristol Long Ashton Bristol BS41 9AF United Kingdom		
State (that is, country) of nationality: GB		State (that is, country) of residence: GB
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)		
State (that is, country) of nationality:		State (that is, country) of residence:
<input type="checkbox"/> Further applicants are indicated on a continuation sheet.		

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCEThe following person is ☒ agent ☐ common representativeand ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DEAN, John Paul
 WITHERS & ROGERS
 Goldings House
 2, Hays Lane
 LONDON SE1 2HW
 United Kingdom

Telephone No.:

+44 117 925 3030

Facsimile No.:

+44 117 925 3530

Teleprinter No.:

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION****Statement concerning amendments:***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filedthe description ☐ as originally filed☐ as amended under Article 34the claims ☐ as originally filed☐ as amended under Article 19 (together with any accompanying statement)☐ as amended under Article 34the drawings ☐ as originally filed☐ as amended under Article 342. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination:☐ which is the language in which the international application was filed.☐ which is the language of a translation furnished for the purposes of international search.☐ which is the language of publication of the international application.☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.**Box No. V ELECTION OF STATES**The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | |
|--|---|--------|
| 1. translation of international application | : | sheets |
| 2. amendments under Article 34 | : | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | sheets |
| 5. letter | : | sheets |
| 6. other (<i>specify</i>) | : | sheets |

For International Preliminary Examining Authority use only

received not received

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

The demand is also accompanied by the item(s) marked below:

- | | |
|--|---|
| 1. <input type="checkbox"/> fee calculation sheet | 4. <input type="checkbox"/> statement explaining lack of signature |
| 2. <input type="checkbox"/> separate signed power of attorney | 5. <input type="checkbox"/> nucleotide and or amino acid sequence listing in computer readable form |
| 3. <input type="checkbox"/> copy of general power of attorney; reference number, if any: | 6. <input type="checkbox"/> other (<i>specify</i>): |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

.....
DEAN, John Paul

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

- | | |
|--|---|
| 3. <input type="checkbox"/> The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. | <input type="checkbox"/> The applicant has been informed accordingly. |
| 4. <input type="checkbox"/> The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5. | |
| 5. <input type="checkbox"/> Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82. | |

For International Bureau use only

Demand received from IPEA on:

REC'D 06 JUL 2001

WIPO

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14


Applicant's or agent's file reference JPD/SHM/P10281PCT	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB00/01035	International filing date (day/month/year) 20/03/2000	Priority date (day/month/year) 18/03/1999	
International Patent Classification (IPC) or national classification and IPC C12N15/54			
Applicant THE UNIVERSITY OF BRISTOL et al.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
 - ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 18/10/2000	Date of completion of this report 04.07.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Ury, A Telephone No. +49 89 2399 8411



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01035

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-27 as originally filed

Claims, No.:

1-40 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

Sequence listing part of the description, pages:

22-27, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/01035

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	13-15 and 29-31, 35 part.
	No:	Claims	1-12, 16-28, 32-34, 36-40
Inventive step (IS)	Yes:	Claims	29-31, 35 part.
	No:	Claims	1-28, 32-34, 36-40
Industrial applicability (IA)	Yes:	Claims	1-37
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB00/01035

Item I.

The numbering of the SEQ ID as originally filed does not correspond to the numbering of the SEQ ID furnished subsequently to this authority in written form with the letter dated 25.05.00 (e.g. SEQ ID 1 as originally filed corresponds to SEQ ID 7 furnished subsequently to this authority in written form (25.05.00).

Since the ISR was carried out on the basis of the sequence listing furnished subsequently to this authority in written form (25.05.00) (see ISR, page 1, item 1.b), this report is also based on the numbering according to the sequence listing furnished subsequently to this authority in written form (25.05.00).

Item V.

Reference is made to the following documents:

D1: DATABASE SWALL [Online] EMBL Heidelberg, Germany; ID: YLF4_CAEEL, AC: Q03574, 1 February 1994 (1994-02-01) WILSON R ET AL.: '2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*' XP002143744

D2: DATABASE EMINV [Online] EMBL Heidelberg, Germany; ID: CEC40H1, AC: Z19154, 27 December 1992 (1992-12-27) BERKS M: 'Caenorhabditis elegans cosmid C40H1' XP002143745

- I) D1 discloses a *C. elegans* protein having an amino acid sequence identical to that shown in SEQ ID 15 of the present application. Thus, D1 destroys the novelty of claims 1-12 (Article 33.2 PCT).
- II) No technical feature distinguishes an engineered organism (e.g. *C. elegans*) engineered to express a polypeptide according to SEQ ID 15 from the naturally occurring organism. Thus, claim 18 lacks novelty over any wild type *C. elegans*.

The same reasoning applies for present claims 21-24 which also lack novelty (Article 33.2 PCT).

- III) Polyunsaturated fatty acids (PUFAs) were known in the art as well as their use in pharmaceutical and nutritive compositions (present description, second paragraph). This destroys the novelty of present claims 32-34 and 36-40.
- IV) The synthesis of PUFAs was thought to be catalysed in a variety of organisms by a specific fatty acid elongase enzyme (present description, third paragraph). Thus, a claim directed to a polypeptide encoding a PUFA elongase from a given organism, without indicating the specific sequence of said PUFA elongase, merely consists of a paraphrase of the technical problem. There is no inventivity in formulating the problem to be solved as a solution. Therefore, claims 13-14 do not fulfil the requirements of Article 33.3 PCT.
- The same reasoning applies for present claim 16 and 17 (with respect to the "variants").

In view of this basic knowledge of the skilled person (i.e. synthesis of PUFAs is catalysed in a variety of organisms by a specific fatty acid elongase enzyme) and given the remark made under paragraph II above, novelty of claims 19, 20 and 25-28 cannot be acknowledged (Article 33.2 PCT).

- V) It would seem that the nucleotide sequence disclosed in D2 is prejudicial to the novelty of SEQ ID 7. Claims 16 and 17, would then lack novelty over D2 (Article 33.2 PCT).
- VI) Claims 29-31 and 35 seem to be novel and inventive (but only when they refer to the specific sequence SEQ ID 15 in claim 3) because the identification of SEQ ID15 (disclosed in D1) as a PUFA elongase was apparently not derivable in an obvious manner from the prior art cited in the ISR.
- VII) For the assessment of the present claims 38-40 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB00/01035

Item VI.

Certain published documents (Rule 70.10)

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO 00/12720	09.03.00	30.08.99	02.09.98

Item VIII.

- 1) It is clear from the description that the following features are essential to the definition of the invention:

- (1) SEQ ID 15 and/or
- (2) SEQ ID 7

Since no independent claim contains these features it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(a) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

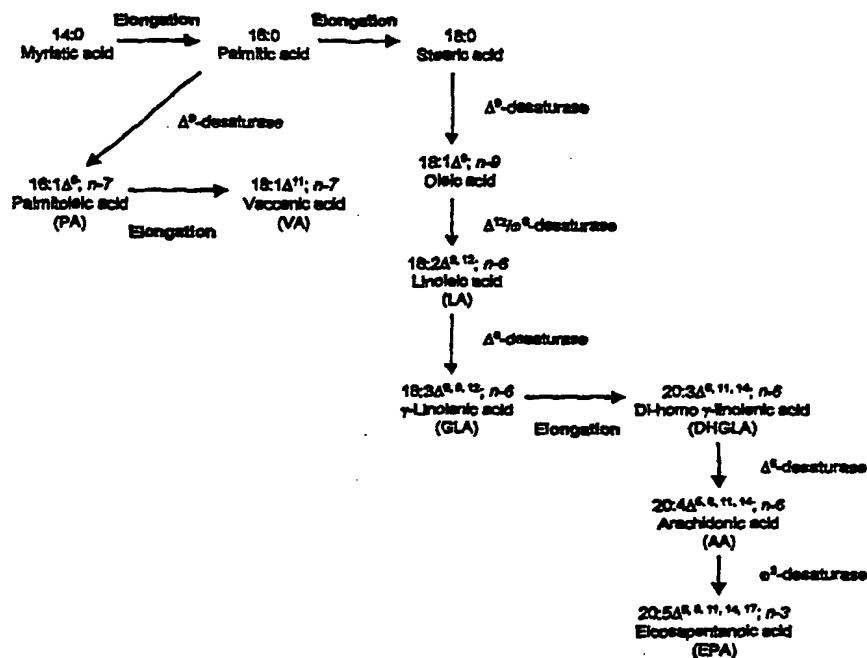
- 2) The terms "at least a portion", "variants (thereof)", "homology" used in claims 3, 4-6, 17 are vague and unclear and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).
- 3) The expression "as herein defined" (claim 1) is not acceptable (Rule 6.2.a PCT).
- 4) Claims 18, 19, 21-23 include **humans** and **human embryos** in their scope. This subject-matter is considered by the present IPEA to be contrary to morality and hence not allowable under Rule 9.1 (i)(ii) PCT.



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁷ : C12N 15/54, 9/10, 1/21, A01H 5/00, C07C 57/03, 57/12, A61K 38/45, 31/202, A23L 1/30</p>	A1	<p>(11) International Publication Number: WO 00/55330</p> <p>(43) International Publication Date: 21 September 2000 (21.09.00)</p>
<p>(21) International Application Number: PCT/GB00/01035</p> <p>(22) International Filing Date: 20 March 2000 (20.03.00)</p> <p>(30) Priority Data: 9906307.5 18 March 1999 (18.03.99) GB 0003869.5 18 February 2000 (18.02.00) GB</p> <p>(71) Applicant (for all designated States except US): THE UNIVERSITY OF BRISTOL [GB/GB]; Senate House, Tyndall Avenue, Bristol BS8 1TH (GB).</p> <p>(72) Inventor; and (75) Inventor/Applicant (for US only): NAPIER, Johnathan, A. [GB/GB]; IACR - Long Ashton Research Station, Dept. of Agriculture Sciences, University of Bristol, Long Ashton, Bristol BS41 9AF (GB).</p> <p>(74) Agents: DEAN, John, Paul et al.; Withers & Rogers, Goldings House, 2 Hays Lane, London SE1 2HW (GB).</p>		<p>(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>

(54) Title: POLYSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS



(57) Abstract

A isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase.

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POLYSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS

The present invention relates to polyunsaturated fatty acid (PUFA) elongases. More specifically, the invention relates to a DNA sequence from *C. elegans* encoding a PUFA elongase.

Unsaturated fatty acids are essential components required for normal cellular function, being involved in a diverse number of roles ranging from membrane fluidity to acting as signal molecules (Gill, I., Valivety, R. (1997). *Trends Biotechnol.* 15, 401-409; Broun, P., *et al* (1999) *Ann. Rev. Nutr.* 19, 197-216). In particular, the class of fatty acids known as the polyunsaturated fatty acids (PUFAs) has attracted considerable interest as pharmaceutical and nutraceutical compounds (Broun *supra*; Horrobin, D. F. (1990) *Reviews in Contemp Pharmacotherapy* 1, 1-45).

The synthesis of PUFAs i.e. fatty acids of 18 carbons or more in length and containing two or more double bonds, is thought to be catalyzed in a variety of organisms by a specific fatty acid elongase enzyme. This elongase is responsible for the addition of 2 carbon units to an 18 carbon PUFA, resulting in a 20 carbon fatty acid. An example of this reaction is the elongation of γ -linolenic acid (GLA; $18:3\Delta^{6,9,12}$) to di-homo- γ -linolenic acid (DHGLA; $20:3\Delta^{8,11,14}$) in which the tri-unsaturated 18 carbon fatty acid is elongated by the addition of a two carbon unit to yield the tri-unsaturated 20 carbon fatty acid. Since there is considerable interest in the production of long chain PUFAs of more than 18 carbons in chain length, for example arachidonic acid and eicosapentanoic acid, the identification of this enzyme is of both academic and commercial interest.

At present, there are no examples of identified cloned genes encoding PUFA elongases, though a number of genes encoding enzymes likely to be involved in other aspects of lipid synthesis have been identified. For example, an *Arabidopsis* gene (FAE1) has been shown to be required for the synthesis of very long chain monounsaturated fatty acids (such as erucic acid; $20:1\Delta^{11}$) (James, D. W. *et al*, (1995) *Plant Cell* 7, 309-319). However, it is clear that this enzyme does not recognize di- and tri-unsaturated 18 carbon fatty acids, for example, linoleic acid, $18:2\Delta^{9,12}$ or α -linolenic acid, $18:3\Delta^{9,12,15}$ respectively, as substrates,

and is therefore not involved in the synthesis of long chain PUFAs (Millar & Kunst (1997), *Plant Journal* **12**, 121-131). This in itself is not surprising, since, of the plant kingdom, only a very few lower plant species, such as the moss *Physcomitrella patens* (Girke *et al.*, (1998), *Plant J*, **15**: 39-48); are capable of synthesising long chain PUFAs, and therefore *Arabidopsis* would not be expected to contain any such enzymes (Napier *et al.* (1997), *Biochem J*, **328**: 717-720; Napier *et al.*, (1999) *Trends in Plant Sci* **4**, 2-5).

A schematic diagram representing a generalized pathway for the product of PUFAs is shown in Figure 1. Biochemical characterisation of mammalian elongation systems (most notably from liver microsomes) has indicated that a mammalian elongase consists of four subunits, made up of a condensing enzyme, a β -ketoreductase, a dehydrase and an enoyl reductase (reviewed in Cinti, D. L., *et al* (1992) *Prog. Lipid Res.* **31**, 1-51). The *Arabidopsis FAE1* gene product encodes a polypeptide of 56kDa, which shows very limited homology to condensing enzymes such as chalcone synthase and stilbene synthase (James, D. W. *supra*). Although *FAE1* is normally only expressed in seed tissues, ectopic expression in non-seed tissue (or heterologously in yeast) revealed that *FAE1* could direct the synthesis of erucic acid (Millar, A. A., Kunst, L. (1997) *Plant J.* **12**, 121-131).

Three fatty acid elongase activities have been characterised from the yeast *S. cerevisiae*. Again, this organism does not synthesis PUFAs, and therefore does not contain genes encoding a PUFA elongase. One gene ELO1, was identified on the basis of a screen to isolate mutants defective in elongation of 14 carbon (i.e. medium) chain saturated fatty acids (Toke & Martin (1996) *J Biol Chem* **271**, 18413-18422). Complementation of *elo1* mutants restored viability, and the ELO1 gene product was shown to encode a polypeptide which was responsible for the specific elongation of 14:0 fatty acids to 16:0 fatty acids.

Two related genes were also detected in the genome of *S. cerevisiae*, and their function determined by disruption. These two genes, subsequently named ELO2 and ELO3, were shown to be involved in the elongation of the very long chain saturated fatty acids found in sphingolipid molecules (Oh *et al* (1997), *J. Biol Chem* **272**, 17376-17384). In particular, ELO2 was required for elongation of fatty acids up to 24 carbons, and ELO3 was required for elongation of the 24 carbon fatty acid to 26 carbons. However, neither gene was

essential for viability. Examination of these three fatty acid elongases revealed the presence of a conserved "histidine box" motif (Shanklin *et al.*, (1994), *Biochemistry*, **33**, 12787-12794) (His-X-X-His-His, where X is any amino acid) towards the centre of the polypeptide sequences. Importantly, there was no detectable homology between the yeast elongases (ELO1,2,3) and the plant very long chain mono-unsaturated fatty acid elongase (FAE1) (Oh *et al. supra*).

In order to identify genes encoding PUFA elongases, it is necessary to study systems in which the synthesis of PUFAs is well documented; a good example of this is the model animal system *C. elegans*, a small free-living worm (Tanaka *et al.*, (1996), *Lipids* **31**, 1173-1178). *C. elegans*, like most other animals, and in contrast to higher plants, synthesises PUFAs such as arachidonic acid (AA; 20:4 $\Delta^{5,8,11,14}$) as precursors to a class of molecules known as the eicosanoids, which in turn serve as precursors for compounds such as prostaglandins and leucotrienes (Horrobin, (1990), *Reviews in Contemp Pharmacotherapy*, 1:1-45). The presence of AA and other long chain polyunsaturated fatty acids in *C. elegans* is well documented (Tanaka *et al.*, (1996), *Lipids* **31**, 1173-1178). The complete sequence of the nematode's genome is now publicly available (*The C. elegans consortium*, 1998, *Science* **282**, 2012-2018; Database at http://www.sanger.ac.uk/Projects/C_elegans/blast_server.shtml).

An object of the invention is to provide an isolated PUFA elongase.

Using the above-mentioned *C. elegans* genomic sequence, together with suitable search strings, the inventors identified eight related putative open reading frames (ORFs) encoding for PUFA elongases. A number of different search criteria were applied to identify a number of (ORFs) which were likely to encode polypeptides with fatty acid elongase activities. These ORFs were then subject to functional characterisation by heterologous expression in yeast, allowing the identification of a PUFA elongase.

Accordingly, a first aspect of the invention provides an isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase i.e. the polypeptide has the function of extending the chain length of an 18 carbon PUFA to 20 carbons in length.

This polypeptide can be used to elevate PUFA levels in animals, thereby providing a ready source of PUFAs.

The polypeptide may be from a eukaryote.

The polypeptide may comprise at least a portion of the amino acid shown in SEQ ID. 15, or variants thereof.

For the purposes of the present application, the term "variant" in relation to a certain sequence means a protein or polypeptide which is derived from the sequence through the insertion or deletion of one or more amino acid residues or the substitution of one or more amino acid residues with amino acid residues having similar properties, e.g. the replacement of a polar amino acid residue with another polar amino acid residue, or the replacement of a non-polar amino acid residue with another non-polar amino acid residue. In all cases, variants must have an elongase function as defined herein.

A second aspect of the invention provides a polypeptide having at least 60 % homology to a polypeptide according to a first aspect of the invention. The polypeptide may have at least 80%, or as much as 90% or more homology to a polypeptide according to a first aspect of the invention.

The polypeptide according to either aspect of the invention may include a sequence motif responsible for Endoplasmic Reticulum (ER) - retention. This allows the polypeptide to be specifically located or targeted to the ER of a cell.

The polypeptide may also be able to elongate palmitoleic acid (PA; 16:1 Δ^9) to vaccenic acid (VA; 18:1 Δ^{11}). Thus, the polypeptide is also capable of elongation of a Δ^9 - monounsaturated 16C fatty acid.

Preferably, the polypeptide is from an animal, more preferably, the animal is an invertebrate such as a worm. Where the animal is a worm, it is preferably *C. elegans*. Alternatively, the animal is a vertebrate, preferably a mammal such as a human, rat or mouse.

A third aspect of the invention provides an isolated DNA sequence, preferably a cDNA sequence, encoding a polypeptide according to a first or second aspect of the invention. This DNA sequence may be used to engineer transgenic organisms.

Preferably, the DNA sequence comprises the sequence shown in SEQ ID NO: 7 or variants of that sequence due, for example, to base substitutions, deletions, and/or additions.

A fourth aspect of the invention provides an engineered organism, such as a transgenic animal, engineered to express a polypeptide according to a first or second aspect of the invention. The engineered organism may be engineered to express elevated levels of the polypeptide, thereby providing a supply of polypeptide at a reduced cost as a reduced number of organisms need be used.

Preferably, the engineered organism is a mammal such as a rat, mouse or monkey.

A fifth aspect of the invention provides an engineered organism containing a synthetic pathway for the production of a polypeptide according to a first or second aspect of the invention. This has the advantage of allowing greater control over the production of PUFAs by the pathway by an organism.

The pathway may include Δ^5 -fatty acid desaturase, and/or Δ^6 -fatty acid desaturase.

The engineered organism according to a fourth or fifth aspect of the invention may be a lower eukaryote, such as yeast. Alternatively, the transgenic organism may be a fish.

A sixth aspect of the invention provides a transgenic plant engineered to express a polypeptide according to a first aspect of the invention.

A seventh aspect of the invention provides a transgenic plant containing a DNA sequence according to a third aspect of the invention.

An eighth aspect of the invention provides a method of producing a PUFA comprising carrying out an elongase reaction catalysed by a polypeptide according to a first or second aspect of the invention.

The PUFA may be di-homo-gamma-linoleic acid ($20:3\Delta^{8,11,14}$), arachidonic acid ($20:4\Delta^{5,8,11,14}$), eicosapentanoic acid ($20:5\Delta^{5,8,11,14,17}$), docosatrienoic acid ($22:3\Delta^{3,16,19}$), docosatetraenoic acid ($22:4\Delta^{7,10,13,16}$), docosapentaenoic acid ($22:5\Delta^{7,10,13,16,19}$) or docosaheptaenoic acid ($22:6\Delta^{4,7,10,13,16,19}$).

The PUFA may be a 24 carbon fatty acid with at least 4 double bonds.

A ninth aspect of the invention provides a PUFA produced by a method according to an eighth aspect of the invention.

The PUFA may be used in foodstuffs, dietary supplements or pharmaceutical compositions.

A tenth aspect of the invention provides a foodstuff comprising a PUFA according to a fifth aspect of the invention. The foodstuff can be fed to an animal.

An eleventh aspect of the invention provides a dietary supplement comprising a PUFA according to a fifth aspect of the invention. The dietary supplement can be supplied to an animal to augment its PUFA levels.

An twelfth aspect of the invention provides a pharmaceutical composition comprising a polypeptide according to a first or second aspect of the invention or a PUFA according to a ninth aspect of the invention.

Preferably, the pharmaceutical composition comprises a pharmaceutically-acceptable diluent, carrier, excipient or extender. This allows the composition to be supplied in a form which best suits the pharmaceutical application in question. For example, a topical application would preferably be a cream or lotion, whereas if the composition was to be ingested a different form would be more suitable.

A thirteenth aspect of the invention provides a method of treatment of an animal, such as a mammal, or a plant, comprising supplying to the animal or plant a DNA sequence according to a third aspect of the invention, a foodstuff according to a tenth aspect of the invention, a dietary supplement according to an eleventh aspect of the invention, a pharmaceutical composition according to a twelfth aspect of the invention or a PUFA according to a ninth aspect of the invention.

Preferably, the mammal is a human.

The invention will now be further described, by way of example only, with reference to SEQ ID1 to 16, and Figures 2 to 11, in which;

SEQ ID1 to 8 show the putative ORFs encoding PUFA elongases A to H respectively; and

SEQ ID9 to 16 show the deduced amino acid sequences of the putative ORFs of SEQ ID NO: 1 to 8 respectively; and

Figures 2 to 9 show hydrophobicity plots for each of PUFA elongases A to H respectively.

Figure 10 shows an amino acid sequence line-up comparing the *C. elegans* ORF F56H11.4 (Z68749) with related sequences.

Figure 11 shows chromatograms of fatty acid methyl esters from transformed yeast.

Introduction to general strategy

Initially the *C. elegans* databases were searched for any sequences which showed low levels of homology to yeast ELO genes (*ELO2* and *ELO3*) using the TBLASTN programme. A similar search was carried out using short (20 to 50 amino acid) stretches of ELO genes which were conserved amongst the three ELO polypeptide sequences. *C. elegans* sequences which were identified by this method were then used themselves as search probes, to identify any related *C. elegans* genes which the initial search with the yeast sequences failed to identify. This was necessary because the level of homology between the yeast ELO genes

and any worm genes is always low (see BLAST scores later). To allow for a more sensitive search of worm sequences, a novel approach was adopted to circumvent the major drawback with searches using the BLAST programmes, namely that the search string (i.e. the input search motif) must be longer than 15 characters for the algorithm to work. Thus, if it was desired to search for a short motif (like a histidine box), then the BLAST programme would not be capable of doing this. A complete list of all the predicted ORFs present in the *C. elegans* genome exists as a database called Wormpep, which is freely available from the Sanger WWW site (http://www.sanger.ac.uk/Projects/C_elegans/webace_front_end.shtml). The latest version of Wormpep was down loaded to the hard disc of a Pentium PC, and re-formatted as a Microsoft Word6 document, resulting in a document of about 3,500 pages. This was then searched using the "Search & Replace" function of Word6, which also allows for the introduction of "wildcard" characters into the search motif. So, for example, it is possible to search both for the short text string HPGG, which would identify any predicted worm ORF present in the Wormpep 3,500 page document containing this motif, or alternatively search with HPGX (where X is a wild card character). Clearly, such (manual) searches of a 3,500 page document are extremely time-consuming and demanding, also requiring visual inspection of each and every identified ORF. For example, searching with a motif such as HXXHH identifies in excess of 300 different ORFs. However, by using a number of different short search strings (as outlined below), and combining these with other methods for identifying putative elongase enzymes, a number of candidate ORFs have been identified.

Database search using the FAE1 polypeptide sequence

As a negative control, to demonstrate that the FAE1 gene sequence was unlikely to provide a useful search sequence in the identification of *C.elegans* sequences encoding for PUFA elongases, the GenBank databases (<http://www.ncbi.nlm.nih.gov/Web/Search/index.html>) were searched using the *Arabidopsis* FAE1 polypeptide sequence to identify related genes or expressed sequence transcripts (ESTs). GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (*Nucleic Acid Research* (1998) **26**, 1-7). There are approximately 2,162,000,000 bases in 3,044,000 sequence records as of December 1998. The search was carried out using the BLAST2 (Basic Local Alignment Search Tool) algorithm (Altschul *et al.*, (1990) *J Mol Biol* **215**,403,410) Although a number

of plant ORFs and ESTs were reported as being related, no animal sequences were identified by this search, confirming the observation that FAE1 was unlikely to be a suitable candidate as a search template for PUFA elongases.

Database search using yeast ELO sequences

Using the three yeast fatty acid elongase sequences (ELO 1, 2, 3) as probes, a number of putative ORFs in the DNA of *C. elegans*-derived cosmid sequences which form the *C. elegans* genomic sequence database were identified. Moreover, an extensive and time-consuming search of a downloaded copy of the WormPep database (<ftp://ftp.sanger.ac.uk/pub/databases/wormpep>) using manual search strings in MSWord 6, identified a number of *C. elegans* ORFs which contained presumptive histidine boxes. Wormpep contains predicted proteins from the *Caenorhabditis elegans* genome sequence project, which is carried out jointly by the Sanger Centre in Cambridge, UK and Genome Sequencing Center in St. Louis, USA. The current Wormpep database, Wormpep 16, contains 16,332 protein sequences (7,120,115 residues). Search strings used included [HXXHH], [HXXXHH], [QXXHH] and [YHH]. Comparison of the data from the two different searches indicated a small (<10) number of putative ORFs as candidate elongases. The histidine box motifs are shown in bold in SEQ ID 9 to 16.

Hydrophobicity plot analysis

Since the fatty acid elongase reaction is predicted to be carried out on the cytosolic face of the endomembrane system (Toke & Martin (1996), *supra*; Oh *et al* (1997), *supra*), the putative *C. elegans* ORFs were examined for potential membrane spanning domains, via Kyte & Doolittle hydrophobicity plots (*J. Mol Biol*, (1982), **157**, 105-132). This revealed a number of ORFs with possible membrane-spanning domains, and also indicated a degree of similarity in the secondary-structure of a number of identified ORFs.

Screening for ER-retention signal sequences

The inventors postulated that since fatty acid elongases are expected to be endoplasmic reticulum (ER) membrane proteins, they might be expected to have peptide signals which are responsible for "ER-retention". In the case of ER membrane proteins, this signal often takes the form of a C-terminal motif [K-K-X₂₋₃-Stop], or similar variants thereof (Jackson *et*

al., (1990), *EMBO J.*, 9, 3153-3162). Further sequence analysis of the *C. elegans* putative elongases revealed that 4 ORFs (F41H10.7, F41H10.8, F56H11.4, Y53F4B.c) had C-terminal motifs that exactly matched this search pattern, and that a further 2 ORFs (F11E6.5, C40H1.4) had related sequences. These sequence motifs are underlined in SEQ ID 9 to 13, 15 and 16.

Chromosome mapping

Since the inventors had previously observed that *C.elegans* genes involved in the synthesis of PUFA may exist in tandem (for example the $\Delta 5$ and $\Delta 6$ desaturases required for AA and GLA synthesis, respectively, are < 1 kB apart on chromosome IV (Michaelson *et al.*, (1998), *FEBS Letts* 439, 215-218), the positions of the putative *C. elegans* elongase ORFs were determined using the Sanger Centre's WebAce *C. elegans* server (http://www.sanger.ac.uk/Projects/C_elegans/webace_front_end.shtml). This indicated that two pairs of putative elongases were in close proximity to each other on the *C. elegans* chromosome IV.

F41H10.7 and F41H10.8 were identified as being approximately 10 Kb apart on chromosome IV, and F56H11.3 and F56H11.4 were identified as being approximately 2 Kb apart on chromosome IV.

Putative *C. elegans* fatty acid elongases

The positions of the putative ORFs in the *C. elegans* genome are shown below i.e. chromosome number, and map position in centiMorgans, together with the GenBank database accession numbers.

The designations used employ the same method as used on the Sanger Centre's *C. elegans* database, i.e. ORF C40H1.4 is predicted coding sequence 4 on cosmid C40H1.

<u>Elongase</u>	<u>Cosmid Sanger ID</u> <u>Code</u>	<u>GenBank Acc</u>	<u>Chromosome</u>
A	C40H1.4	Z19154	III

B	D2024.3	U41011	IV, 7.68
C	F11E6.5	Z81058	IV, 18.8
D	F41H10.7*	U61954	IV, 29.8
E	F41H10.8*	U61954	IV, 29.8
F	F56H11.3 [#]	Z68749	IV, 2.5
G	F56H11.4 [#]	Z68749	IV, 2.5
H	Y53F4B.c	Z92860	II

* or [#] indicates genes in tandem

Comparison of *C. elegans* putative elongase ORFs with yeast genes:

Each of the three yeast ELO polypeptides were compared against all of the worm putative elongase translated ORF sequences, and then ranked in order of similarity (as measured by the BLAST score) (Altschul *et al* (1990), *supra*)

The results are shown below, with the ORF sequences ranked from most similar to least similar, and the BLAST scores are shown in brackets:

Yeast ELO1 (14 to 16 carbon fatty acid elongase)

G (262) > E (241) > D (225) > C (219) > A (216) > F (215) > H (197) > B (172)

Yeast ELO2 (24 carbon sphingolipid elongase)

E (231) > C (226) > G (189) > A (181) > F (166) > D (150) > H (141) > B (140)

Yeast ELO3 (24 to 26 sphingolipid elongase)

D (171) > G (163) > F (154) > A (152) > E (150) > C (131) > B (132) > H (128)

It is clear from the numeric values of the BLAST scores that the sequences are related, but the levels of homology are low. For comparison, the BLAST score for homology between two related worm proteins, the $\Delta 5$ and the $\Delta 6$ desaturase is in excess of 500.

Analysis of potential sphingolipid ancestry

Previously, the inventors had noted the similarities between the fatty acid $\Delta 6$ desaturase and sphingolipid desaturases in plants, and that the two distinct enzymes could have arisen from one ancestral gene. Moreover, it was considered likely that the sphingolipid desaturase predated the fatty acid desaturase, and may in fact have been the ancestral progenitor. Therefore it is plausible that the next step in the arachidonic acid biosynthetic pathway has also evolved from the sphingolipid metabolic pathway. It is therefore considered highly significant that some of the *C. elegans* ORF putative elongases have similarity to sphingolipid enzymes. For this reason, these ORFs are considered to be very clear candidates for PUFA elongases. It has previously been considered that the *C. elegans* $\Delta 5$ and $\Delta 6$ fatty acid desaturases have evolved from 1 ancestral gene (Michaelson *et al.*, (1998), *FEBS Letts* 439, 215-218). It is also significant that one pair of *C. elegans* putative elongase ORFs (F & G) genetically maps close to the $\Delta 5/\Delta 6$ fatty acid desaturase genes, with both gene pairs being located at the top end of chromosome IV.

<u>Cosmid Sanger ID</u> <u>Code</u>	<u>GenBank Acc</u>	<u>Chromosome</u>	<u>Encoded Peptide</u>
W08D2.4	Z70271	IV, 3.06	$\Delta 6$ fatty acid desaturase
T13F2.1	Z81122	IV, 3.06	$\Delta 5$ fatty acid desaturase

Cloning of Desaturase and Elongase Genes in Yeast Expression Vectors

Putative elongases sequences F56H11.4 and F41H10.8 were cloned by PCR into the pYES2 vector (Invitrogen). A *C. elegans* mixed stage cDNA library was used as a PCR template. F56H11.4 was amplified using primers:

56h114.for 5'-GCGGGTACCATGGCTCAGCATCCGCTC-3' and;

56h114.rev 5'-GCGGGATCCTTAGTTGTTCTTCTTCTT-3'.

F41H10.8 was amplified using primers:

41h108.for 5'-GCGGGTACCATGCCACAGGGAGAAGTC-3' and;

41h108.rev 5'-GCGGGATCCTTATTCAATTTTCTTTT-3'.

Amplified sequences were then restricted using *KpnI* and *BamHI* (underlined in the forward and reverse primers, respectively), purified using the Qiagen PCR purification kit, and ligated into a *KpnI/BamHI* cut pYes2 vector.

An ORF encoding the *Mortierella alpina* Δ^5 -fatty acid desaturase (Michaelson, L. V., *et al* (1998) *J. Biol. Chem.* **273**, 19055-19059) was amplified using primers:

Mad5.for 5'-GCGAATTCACCATGGGTACGGACCAAGGA-3' and;

Mad5.rev 5'-GCGGAGCTCCTACTCTTCCTTGGGACG-3',

and restricted using *EcoRI* and *SacI*, gel purified as described and ligated into a *EcoRI/SacI* cut pESC-TRP vector (Stratagene) to generate pESC/ Δ^5 .

An ORF encoding the borage Δ^6 -fatty acid desaturase (Sayanova, O., *et al* (1997) *Proc. Natl. Acad. Sci USA* **94**, 4211-4216) was restricted from pGEM3 using *BamHI* and *XhoI* and ligated into a *BamHI/XhoI* cut pESC-TRP vector to generate pESC/ Δ^6 .

A double construct was also generated by ligating the *Bam*HI/*Xho*I borage Δ^6 insert into the pESC/ Δ^5 construct described previously, generating pESC/(Δ^5, Δ^6).

Functional Characterisation in Yeast

Elongases and desaturase constructs were introduced in *Saccharomyces cerevisiae* W303-1A using a lithium acetate based method (Elble, R. (1992) *Biotechniques* 13, 18-20) and expression of the transgenes was induced by addition of galactose to 2% (w/v) as described in Napier *et al* (Napier, J. A., *et al* (1998) *Biochem J* 330, 611-614; Michaelson L. V., *supra*; Michaelson, L. V., (1998) *FEBS Letts* 439, 215-218). Yeast transformants containing pYES2-derived constructs were grown on synthetic minimal media (SD, the composition of which is defined in Sherman, F (1991) *Methods in Enzymology* 194, 3-21); synthetic minimal medium minus uracil; pESC-derived constructs were grown on SD minimal medium minus tryptophan. Co-transformed yeast (containing both pYES2 and pESC derivatives) were grown on SD minimal medium minus uracil and tryptophan. Prior to induction, cultures were grown in the presence of 2% raffinose and supplemented with 0.5 mM of the appropriate fatty acid substrate in the presence of 1% tergitol-(NP40) (Sigma). All cultures were then grown for a further 48-h unless indicated.

Fatty Acid Analysis

To identify the elongation reaction responsible for the synthesis of di-homo- γ -linolenic acid (DHGLA; 20:3 $\Delta^{8,11,14}$) from GLA, this latter fatty acid was supplied as the (exogenous) substrate.

Lipids were extracted from transformed and control yeast by homogenisation in MeOH-CHCl₃ using a modification of the method of Bligh and Dyer (Dickenson & Lester (1999) *Biochim Biophys Acta* 1426, 347-357). The resulting CHCl₃ phase was evaporated to dryness under nitrogen gas and the samples were transmethylated with 1M HCl in methanol at 80 °C for 1 hour. Fatty acid methyl esters (FAMES) were extracted in hexane and purified using a small column packed with Florisil. Analysis of FAMES was conducted using a Hewlett Packard 5880A Series Gas Chromatograph equipped with a 25M x 0.32mm RSL-500BP bonded capillary column and a flame ionisation detector. Fatty acids were identified by comparison of retention times with FAME standards (Sigma)

separated on the same GC. Quantitation was carried out using peak height area integrals expressed as a total of all integrals (Bligh, E.G. & Dyer, W.J. (1959) *Can. J. Biochem. Physiol.* 37, 911-917).

Total fatty acids extracted from yeast cultures were analysed by gas chromatography (GC) of methyl ester derivatives. Lipids were extracted, transmethyated and the fatty acid methyl esters (FAMES) analysed as described by Sayanova *et al.*

Figure 11 shows chromatograms of fatty acid methyl esters from yeast transformed with the control (empty) plasmid pYES2 (Fig. 11A) or with ORF F56H11.4 in pYES2 (Fig. 11B). Exogenous substrate in the form of GLA was supplied to the cultures. Two novel peaks are observed in (B); these peaks (annotated as 20:3 and 18:1*) were identified (against known standards) as DHGLA and vaccenic acid, respectively. Detection was by flame ionisation.

One cDNA ORF tested in this manner displayed a high level of elongase activity on the GLA substrate, converting 44% to DHGLA. The identity of this elongation product was confirmed as DHGLA by comparison with a known standard (the standards used were known standards for either DHGLA, AA, EPA or VA from Sigma Chemicals, Ltd.), using GCMS analysis using a Kratos MS80RFA (Napier, J. A., *supra*; Michaelson, L. V., *supra*; Michaelson, L. V., *supra*). The deduced amino acid sequence of the functional elongase clone identified it as being encoded by the *C. elegans* gene F56H11.4, and comparison with the yeast *ELO* genes showed low homology confined to a few short amino acid motifs (see Fig. 10). Some similarity with a mouse gene Cig30 (Tvrdik, P., (1997) *J. Biol. Chem.* 272, 31738-31746), which has been implicated in the recruitment of brown adipose tissue in liver tissue, was also observed, as well as a potential human homologue encoded by a gene located on chromosome 4q25, BAC 207d4. The most closely related *C. elegans* ORFs, F41H10.8 (U61954) and F56H11.3 (Z68749) are also shown, as is part of a related human gene present on chromosome IV (present on BAC clone B207d4; AC004050). The GenBank accession numbers are given for all sequences.

The range of fatty acids synthesised by *C. elegans* can potentially require a number of different elongation reactions (Tanaka, T., (1996) *Lipids* 31, 1173-1178). The substrate-specificity of the F56H11.4 PUFA elongase was therefore determined using a

range of exogenously supplied fatty acids. This revealed that GLA is the major substrate, with a number of other fatty acids being elongated at a lower efficiency (see Table 1). Although most of these substrates are polyunsaturated fatty acids, it was unexpectedly observed that palmitoleic acid (PA; 16:1 Δ^9) was also elongated by F56H11.4 to yield vaccenic acid (VA; 18:1 Δ^{11}). The biosynthetic pathway for VA is unclear, but the data indicate that it may be synthesised by elongation of Δ^9 -monounsaturated 16C fatty acid.

The *C. elegans* PUFA elongase ORF F56H11.4 maps to the top of chromosome IV (at 4.32 cM) with a related sequence (F56H11.3; 51 % similarity) located 1,824bp downstream. Another *C. elegans* gene (F41H10.8) was also observed, which is present on chromosome IV, and which shows a slightly higher level (53%) of similarity to the PUFA elongase than F56H11.3 (see Fig. 10). However, when a PCR product encoding ORF F41H10.8 was expressed in yeast in a manner identical to that used for F56H11.4, the former failed to direct the elongation of any fatty acids, despite the provision of a range of substrates (see Table II).

In order to reconstitute the PUFA biosynthetic pathway in a heterologous system, the PUFA elongase F56H11.4 was expressed in yeast in conjunction with either the Δ^6 - or Δ^5 -fatty acid desaturases previously isolated and characterised by the inventor (Napier, J. A., *supra*; Michaelson, L. V., *supra*). Expression of the Δ^6 -fatty acid desaturase and F56H11.4 was carried out in the presence of two different substrates (LA or ALA) while the Δ^5 -fatty acid desaturase and the elongase were expressed in the presence of GLA only. This demonstrated that was possible to combine a desaturase and an elongase in yeast to generate significant amounts of a final "product" (see Table III). In the case of the elongase and the Δ^6 -fatty acid desaturase, the reactions proved highly efficient with the production of 4.5% of DHGLA from the LA substrate. This resulted from 25% desaturation of the LA substrate to GLA, which was then elongated to DHGLA at a similar level of efficiency (18%). This is lower than the % conversion observed for GLA when supplied exogenously (see Table I), indicating that the *in vivo* production of substrates for elongation may be rate-limiting.

If ALA was used as a substrate, 27% of this was initially Δ^6 -desaturated to yield octadecatetraenoic acid (OTA; 18:4 $\Delta^{6,9,12,15}$) but only 8% of was subsequently elongated to yield eicosatetraenoic acid (20:4 $\Delta^{8,11,14,17}$). Thus, the conversion efficiency of ALA to the final 20-carbon tetraenoic PUFA was only about 2.2%.

Since DHGLA is an *n*-6 fatty acid, whilst the OTA-derived eicostetraenoic acid is an *n*-3 type, this demonstrates that the elongase is capable of accepting both forms of essential fatty acid, albeit with different efficiencies. Verification was also provided that the 20C PUFAs synthesised in the yeast expression system were generated by the Δ^6 -desaturation of 18C substrates which were subsequently elongated, as the Δ^6 -desaturase showed no activity on 20:2 or 20:3 substrates (see Table III).

The combination of the Δ^5 -desaturase and the elongase also demonstrated that these two enzymes could work in tandem, although the efficiency of this overall conversion was lower (3.3% AA from GLA) which was due to the previously observed low activity of the Δ^5 -desaturase enzyme itself (Michaelson, L. V., *supra*; Michaelson, L. V., *supra*). Thus, although nearly 45% of the GLA substrate was elongated to DHGLA, only 7.5% of this was then desaturated to AA (see Table III).

Finally, the production of either AA or eicosapentanoic acid (EPA; 20:5 $\Delta^{5,8,11,14,17}$) in yeast from dienoic or trienoic 18 carbon substrates was achieved via expression of all three enzymes (the two desaturases and the F56H11.4 PUFA elongase) simultaneously. As shown in Table IV, small but significant amounts of AA were produced when the yeast was supplied with the 18C dienoic fatty acid LA.

GC-Mass Spectroscopy (MS) Analysis

Peak identification and confirmation were carried out by GC-MS using a Kratos MS80RFA using known standards (Sigma). The identity of this 20C PUFA was verified by GCMS, indicating that the conversion efficiency from LA was 0.65%. When ALA was used as a substrate, 12.5% of the (Δ^6 -desaturated and elongated) eicosatetraenoic *n*-3 fatty acid was Δ^5 -desaturated, resulting in a total conversion of 0.3% of the ALA substrate to EPA (the identity of EPA was confirmed by GCMS).

Expression of *C. elegans* elongase in plants

In order to express *C. elegans* elongase in plants, the following protocol is an example of a process which can be used to create the transgenic plants. *C. elegans* ORF sequence can be subcloned into a plant expression vector pJD330, which comprises a viral 35S promoter, and a Nos terminator. The resulting cassette or promoter/coding sequence/terminator can then be subcloned into the plant binary transformation vector pBin 19, and the resulting plasmid introduced into *Agrobacterium tumefaciens*. This *Agrobacterium* strain can then be used to transform *Arabidopsis* by the vacuum-infiltration of inflorescences, and the seeds harvested and plated onto selective media containing kanamycin. Since pBin 19 confers resistance to this antibiotic, only transformed plant material will grow. Resistant lines can therefore be identified and self-fertilized to produce homozygous material. Leaf material can then be analyzed for expression of *C. elegans* elongase.

Fatty acid methyl ester analysis can be carried out as previously described.

Table I

mole% Fatty Acids
F56E11.4

ORF

(Control)

Substrate	GLA		LA		ALA		EPA
Induction	+	-	+	-	+	-	+
16:0	19.9 ± 3.5	20.5 ± 4.1	27.7 ± 1.4	29.8 ± 0.2	22.9 ± 1.5	23.9 ± 1.0	19.1 ± 0.7
16:1	40.9 ± 3.1	49.4 ± 3.2	32.5 ± 4.4	34.4 ± 1.8	21.2 ± 2.2	24.8 ± 4.9	18.1 ± 1.5
18:0	4.7 ± 0.9	4.9 ± 0.5	5.6 ± 0.5	5.6 ± 0.3	5.1 ± 0.3	4.4 ± 0.1	5.0 ± 0.3
18:1	24.9 ± 1.4	25.2 ± 2.3	16.9 ± 0.9	16.1 ± 0.3	11.2 ± 2.4	10.7 ± 1.5	10.1 ± 1.1
18:1*	9.6 ± 0.6	-	3.9 ± 0.6	-	3.2 ± 0.6	-	3.1 ± 0.4
LA	-	-	-	-	34.4 ± 4.2	36.2 ± 5.6	-
ALA	-	-	-	-	-	-	43.1 ± 3.9
GLA	-	-	7.5 ± 1.2	14.0 ± 0.3	-	-	45.8 ± 4.8
20:2	-	-	-	-	2.0 ± 0.9	-	-
DEGLA	-	-	5.8 ± 0.9	-	-	-	-
20:3	-	-	-	-	-	-	1.5 ± 0.1
EPA	-	-	-	-	-	-	22.8 ± 0.7
% Elongated	-	-	-	-	-	-	20.1 ± 2.4
GLA	-	-	44	-	-	-	-
LA	-	-	-	-	55	-	-
ALA	-	-	-	-	-	34	-
EPA	-	-	-	-	-	0	-

Table II

ORF *mole% Fatty Acids*
F41H10.8

Substrate	GLA		LA		ALA		EPA	
	+	-	+	-	+	-	+	-
Induction	+	-	+	-	+	-	+	-
16:0	19.0±0.9	19.3±0.2	28.1±0.6	28.0±0.9	23.9±0.7	24.4±0.2	22.8±0.2	23.0±0.6
16:1	50.9±0.7	50.8±0.6	33.5±2.2	35.5±1.5	22.4±2.1	23.6±0.3	17.6±0.2	34.7±3.6
18:0	4.2±0.1	5.1±0.1	5.3±0.1	5.6±0.1	5.1±0.2	5.8±0.1	5.4±0.3	4.8±0.7
18:1	24.5±1.3	24.9±0.5	16.2±1.4	17.1±1.0	9.1±0.3	10.1±0.2	7.8±0.1	15.3±2.5
18:1*	ND	-	ND	-	ND	-	ND	ND
LA	-	-	-	-	39.5±0.6	36.1±0.4	-	-
ALA	-	-	-	-	-	-	46.4±0.5	-
GLA	-	-	14.3±1.6	14.2±0.6	-	-	-	-
20:2	-	-	-	-	ND	-	-	-
DHGLA	-	-	ND	-	-	-	-	-
20:3	-	-	-	-	-	-	ND	-
EPA	-	-	-	-	-	-	22.3±2.8	23.8±2.2
% Elongated								
GLA	-	-	0	-	-	-	-	-
LA	-	-	-	0	-	-	-	-
ALA	-	-	-	-	0	-	-	-
EPA	-	-	-	-	-	-	0	-

Table III

Construct	<i>mole% Fatty Acids</i>							
	Δ^6		$F56H11.4 + \Delta^6$				$F56H11.4 + \Delta^5$	
	20:2	20:3	LA		ALA		GLA	
Substrate	+	-	+	-	+	-	+	-
Induction	+	-	+	-	+	-	+	-
16:0	24.7 ± 1.3	25.2 ± 1.5	18.7 ± 0.6	23.7 ± 0.5	17.4 ± 0.7	21.0 ± 1.3	27.9 ± 4.2	29.8 ± 3.8
16:1	46.0 ± 2.8	43.7 ± 3.7	18.9 ± 1.2	24.6 ± 0.7	5.3 ± 0.6	9.1 ± 0.9	24.6 ± 3.4	25.1 ± 3.2
16:2	5.2 ± 1.2	4.1 ± 1.4	0.6 ± 0.1	-	0.4 ± 0.1	-	-	-
18:0	4.8 ± 0.4	5.1 ± 0.4	4.0 ± 0.3	5.1 ± 0.1	6.2 ± 0.7	5.4 ± 0.2	5.6 ± 0.8	5.4 ± 0.7
18:1	15.3 ± 1.1	16.1 ± 1.2	12.2 ± 1.4	11.2 ± 0.4	5.7 ± 0.8	6.0 ± 0.4	12.7 ± 2.9	13.0 ± 2.5
18:1*	-	-	7.7 ± 0.7	-	2.6 ± 0.3	-	2.9 ± 0.9	-
LA	-	-	25.0 ± 3.2	35.4 ± 2.1	-	-	-	-
ALA	-	-	-	-	42.3 ± 3.3	58.5 ± 4.7	-	-
GLA	-	-	7.9 ± 2.2	-	-	-	13.2 ± 3.6	19.2 ± 3.5
OTA	-	-	-	-	15.3 ± 1.8	-	-	-
20:2	4.0 ± 0.3	-	3.3 ± 0.5	-	-	-	-	-
DHGLA	-	-	1.7 ± 0.2	-	-	-	9.8 ± 1.8	-
20:3	-	5.8 ± 0.5	-	-	3.4 ± 0.4	-	-	-
AA	-	-	-	-	-	-	0.8 ± 0.2	-
20:4	-	-	-	-	1.4 ± 0.2	-	-	-
EPA	-	-	-	-	-	-	-	-
% Elongated								
GLA	-	-	17.7	-	-	-	44.5	-
OTA	-	-	-	-	8.4	-	-	-
LA	-	-	8.7	-	-	-	-	-
ALA	-	-	-	-	5.4	-	-	-

SEQ ID1

C40H1.4

atggagcttgccgagttctggaatgatctcaacaccttcaccatctacggaccgaatcac
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ctttattttcattttaattaagggtgattcaaaagtttatggagaatcgaaaaccattcact
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SEQ ID2

D2024.3

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tcaattacagcatctgtcgtgtatgttagccgtcatttttacaggaaagaagggtggtctc
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cgaaaccgaaaatcacttaatagtttctcaaatgtttcagattatggaaaagtacaagccc
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ttcgcaatgagcaaaacttttcgagctcatcgacacaatcttcacgttcttcgtaaacgt
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SEQ ID3

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SEQ ID4

F41H10.7

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SEQ ID5

F41H10.8 (ce477)

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SEQ ID6

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SEQ ID7

F56H11.4 (Ce 166)

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SEQ ID8

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SEQ ID9**A**

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 51 YWYHSITISV LYFILIKVIQ KFMENRKPFT LKYPLILWNG ALAAFSIIAT
 101 LRFSIDPLRS LYAEGFYKTL CYSCNPTDVA AFWSFAFALS KIVELGDTMF
 151 IILRKRLIF **LHYYHHA**AVL IYTVHSGAEH TAAGRFYILM NYFAHSLMYT
 201 YYTVSAMGYR LPKWVSMVT TVQTQMLAG VGITWMVYKV KTEYKLPCQQ
 251 SVANLYLAFV IYVTFAILFI QFFVKAYIIK SSKKSKSVKN E*

SEQ ID10**B**

1 MAKYDYNPKY GLENYSIFLP FETSFDAFRS TTWMQNHWHYQ SITASVVYVA
 51 VIFTGKKVVL IYKKSrvITF ESSLQNAIKN RNRKSLNSSQ MFQIMEKYKP
 101 FQLDTPLFVW NSFLAIFSIL GFLRMTPEFV WSWSAEGNSF KYSICHSSYA
 151 QGVTGFWTEQ FAMSKLFELI DTIFIVLRKR PLIFL**HWYHH** VTVMiyTWHA
 201 YKDHTASGRW FIWMNYGVHA LMYSYYALRS LKFRLLPKQMA MvVTTLQLAQ

251 MVMGVIIGVT VYRIKSSGEY CQQTWDNLGL CFGVYFTYFL LFANFFYHAY
301 VKKNRRTVNY ENNSKNFPDL VLIYLRKKVS RKSKNRQCSE NNYKIQFSSN
351 FVNVDGKKHK KTYELILPRR KMTTILTFLF GKNRIFSKYQ KNRKNISIPV
401 DFEILEPKED INANIAEPSI TTRSAAARRK VQKAD*

SEQ ID11

C

1 MAAQQTSPAA TLVDVLTKPW SLDQTDSYMS TFPVPLSYKIM IGYLVTIYFG
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101 YCQENENYYTD ASTGFWGWAF VMSKAPELGD TMFLVLRKKP VIFMHWYHHA
151 LTFVYAVVTY SEHQAWARWS LALNLAVHTV MYFYFAVRAL NIQTPRPVAK
201 FITTIQIVQF VISCYIFGHL VFIKSADSVF GCAVSWNVLS IGGLMYISYL
251 FLFAKFFYKA YIQKRSPTKT SKQE*

SEQ ID12

D

1 MSSDDRGRTR FKMDQILGT NFTYEGAKEV ARGLEGFSAK LAVGYIATIF
51 GLKYMKDRK AFDLSTPLNI WNGILSTFSL LGFLFTFPTL LSVIRKDGFS
101 HTYSHVSELY TDSTSGYWIF LWVISKIPEL LDTVFIPLRK RPLIFMHWYH
151 HALTGYYALV CYHEDAVHNV WVVWMNYIIH AFMYGYLLK SLKVPIPPSV
201 AQAITTSQMV QFAVAIFAQV HVSYPHYVEG VEGLYSFRG TAIGFFMLTT
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SEQ ID13

E

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YAFMSFEANL GFNTWITWMN FSVHSIMYGY YMLRSFGVKV PAWIAKNITT
MQILQFVITH FILFHVGYLA VTGQSV DSTP GYYWFCLLME ISYVVLFGNF
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SEQ ID14

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51 EVFPHIRARR FIQEHFGLFV QMAIAYVILV FSIKRFMRDR EPFQLTTALR
101 LWNFFLSVFS IYGSWTMFPF MVQQIRLYGL YGCGCEALSN LPSQAEYWL

151 LTILSKAVEF VDTFFLVLRK KPLIFLHWYH HMAFVFFCS NYPTSSQSR
201 VGVIVNLFVH AFMYPYYFTR SMNIKVPAKI SMAVTVLQLT QFMCFIYGCT
251 LMYSLATNQ ARYPSNTPAT LQCLSYTLHL L* -

SEQ ID15**G**

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SILYMVVVFV TKWFMRNRQP FQLTIPLNIW NFILAAFSIA GAVKMTPEFF
GTIANKGIVA SYCKVFDFTK GENGYVWVLF MASKLFELVD TIFLVLRKRP
LMFLHWYHHI LTMIYAWYSH PLTPGFNRYG IYLNFVVHAF MYSYYFLRSM
KIRVPGFIAQ AITSLQIVQF IISCAVLAHL GYLMHFTNAN CDFEPSVFKL
AVFMDTTYLA LEVNFFLQSY VLRGGKDKYK AVPKKKNN*

SEQ ID16**H**

MSAEVSERFKVWTGNNETIIYSPFEYDSTLLIESCRCTYQLLILLRQI
YYRDIWSHGKACDXLLLAWNGFLAVFSIMGTWRFGIEFYDAVFRXG
FIXSICLAVNPRSPSAFWACMFALSKIAEFGDTMFLVLRKRPVIFLHWYHH
AVVLILSWHAAIELTAPGRWFI FMNYLVHSIMYTYYAITSIGYRXPKIVSMT
VTFLQTLQMLIGVSISICVLYLKLNGEMCQQSYDNLALSFGIYASFLVLSFF
NNAYLVKDKKPDVKKD*

Claims

1. An isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase as herein defined.
2. A polypeptide according to claim 1 wherein the polypeptide is from a eukaryote.
3. A polypeptide according to claim 1 or claim 2 wherein the polypeptide has at least a portion of the amino acid sequence shown in SEQ ID 15, or variants thereof.
4. A polypeptide having at least 60% homology to a polypeptide according to claim 3 and having a PUFA elongase function.
5. A polypeptide according to claim 4 having at least 80% homology.
6. A polypeptide according to claim 5 having at least 90% homology.
7. A polypeptide according to any preceding claim wherein the polypeptide sequence includes a sequence motif responsible for Endoplasmic Reticulum (ER) - retention.
8. A polypeptide according to any preceding claim wherein the polypeptide is capable of elongating palmitoleic acid (PA; 16:1 Δ^9) to vacceric acid (VA; 18:1 Δ^{11}).
9. A polypeptide according to any preceding claim wherein the polypeptide is from an animal.
10. A polypeptide according to claim 9 wherein the animal is an invertebrate.
11. A polypeptide according to claim 10 wherein the invertebrate is a worm.
12. A polypeptide according to claim 11 wherein the worm is *C. elegans*.

13. A polypeptide according to claim 9 wherein the animal is a vertebrate.
14. A polypeptide according to claim 13 wherein the vertebrate is a mammal.
15. A polypeptide according to claim 14 wherein the mammal is a human, rat or mouse.
16. A DNA sequence encoding a polypeptide according to any preceding claim.
17. A DNA sequence according to claim 16 wherein the DNA comprises the sequence shown in SEQ ID 7 or variants of that sequence due to base substitutions, deletions and/or additions.
18. An engineered organism engineered to express a polypeptide according to any one of claims 1 to 15.
19. An engineered organism according to claim 18 wherein the animal is a mammal.
20. An engineered organism according to claim 19 wherein the mammal is a rat, mouse or monkey.
21. An engineered organism containing a synthetic pathway for the production of a polypeptide according to any one of claims 1 to 15.
22. An engineered organism according to claim 21 wherein the pathway includes Δ^5 -fatty acid desaturase.
23. An engineered organism according to claim 21 or 22 wherein the pathway includes Δ^6 -fatty acid desaturase.
24. An engineered organism according to any one of claims 21 to 23 wherein the animal is a lower eukaryote.

25. An engineered organism according to claim 24 wherein the lower eukaryote is a yeast.
26. An engineered organism according to claim 18 wherein the animal is a fish.
27. A transgenic plant engineered to express a polypeptide according to any one of claims 1 to 15.
28. A transgenic plant containing a DNA sequence according to claim 16 or 17.
29. A method of producing a PUFA comprising carrying out an elongase reaction catalysed by a polypeptide according to any one of claims 1 to 15.
30. A method according to claim 29 wherein the PUFA is di-homo-gamma-linoleic acid ($20:3\Delta^{8,11,14}$), arachidonic acid ($20:4\Delta^{5,8,11,14}$), eicosapentanoic acid ($20:5\Delta^{5,8,11,14,17}$), docosatrienoic acid ($22:3\Delta^{3,16,19}$), docosatetraenoic acid ($22:4\Delta^{7,10,13,16}$), docosapentaenoic acid ($22:5\Delta^{7,10,13,16,19}$) or docosahexaenoic acid ($22:6\Delta^{4,7,10,13,16,19}$).
31. A method according to claim 29 wherein the PUFA is a 24 carbon fatty acid with at least 4 double bonds.
32. A PUFA produced by a method according to any one of claims 29 to 31.
33. A foodstuff comprising a PUFA according to claim 32.
34. A dietary supplement comprising a PUFA according to claim 32.
35. A pharmaceutical composition comprising a polypeptide according to any one of claims 1 to 15.
36. A pharmaceutical composition comprising a PUFA according to claim 32.

37. A pharmaceutical composition according to claim 35 or claim 36 wherein the composition comprises a pharmaceutically-acceptable diluent, carrier, excipient or extender.
38. A method of elevating the PUFA levels of an animal or a plant by supplying to the animal or plant a polypeptide according to any of claims 1 to 15, a DNA sequence according to claim 16 or claim 17, a foodstuff according to claim 33, a dietary supplement according to claim 34, a pharmaceutical composition according to any of claims 35 to 37 or a PUFA according to claim 32.
39. A method of treatment according to claim 38 wherein the animal is a mammal.
40. A method of treatment according to claim 39 wherein the mammal is a human.

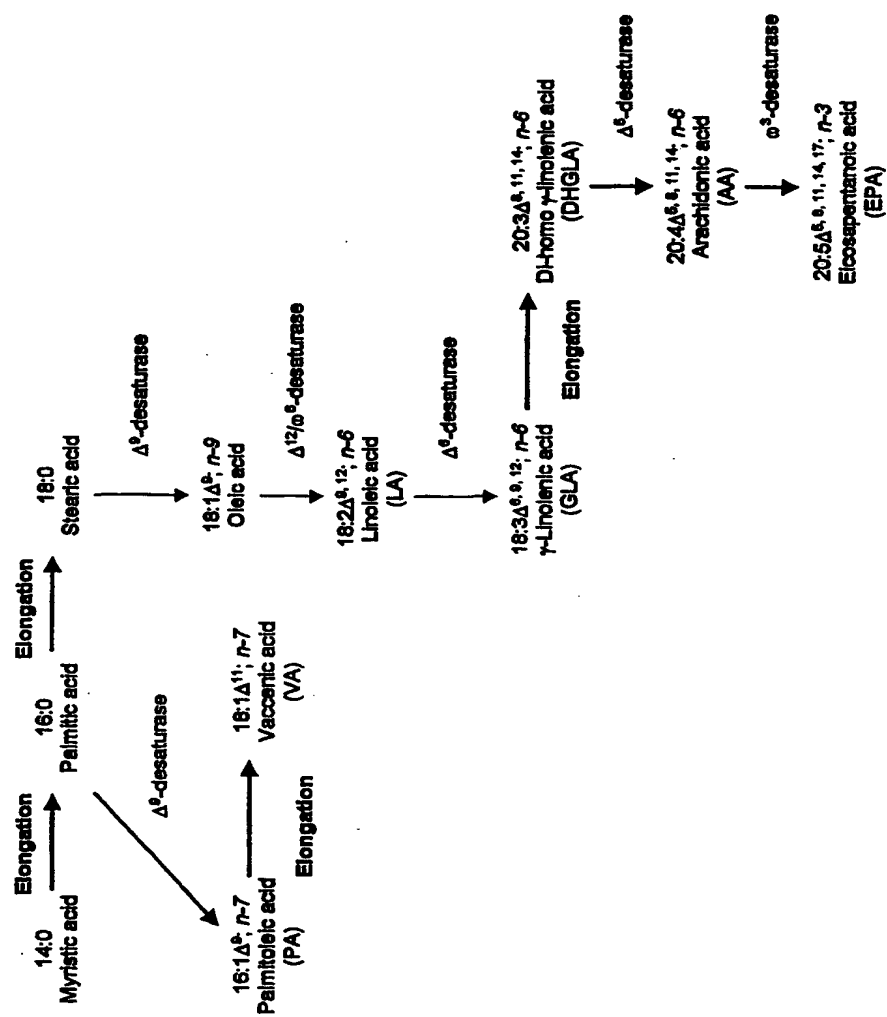


FIG. 1

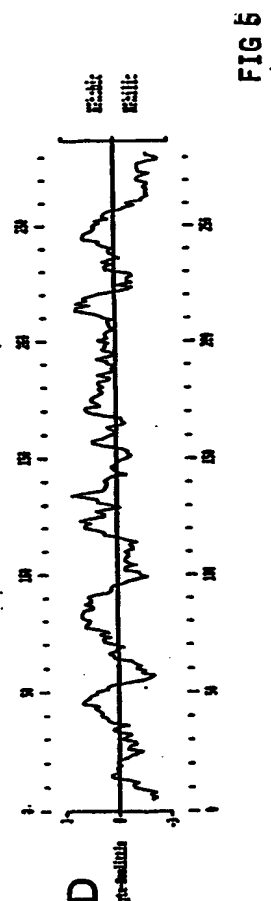
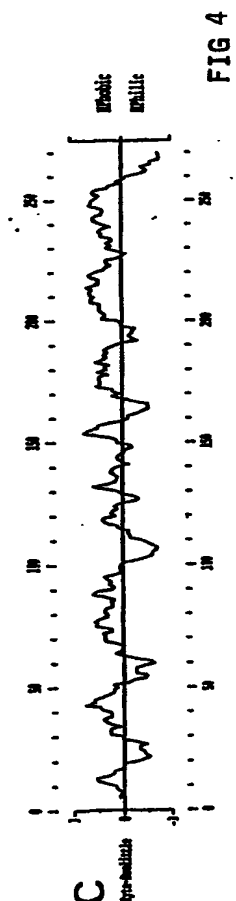
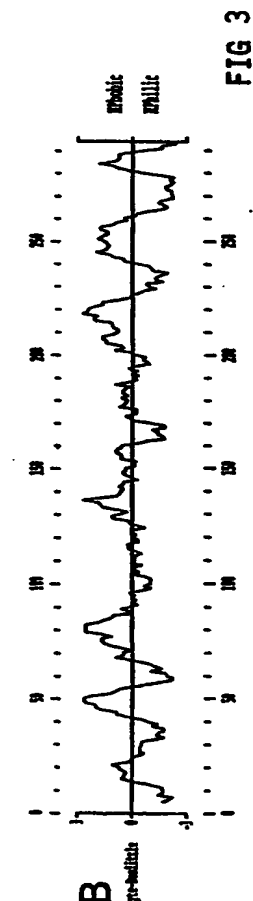
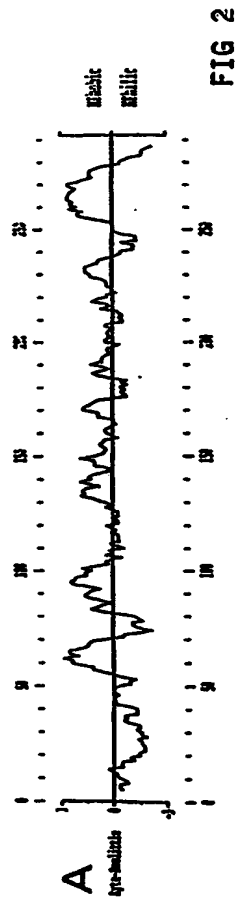
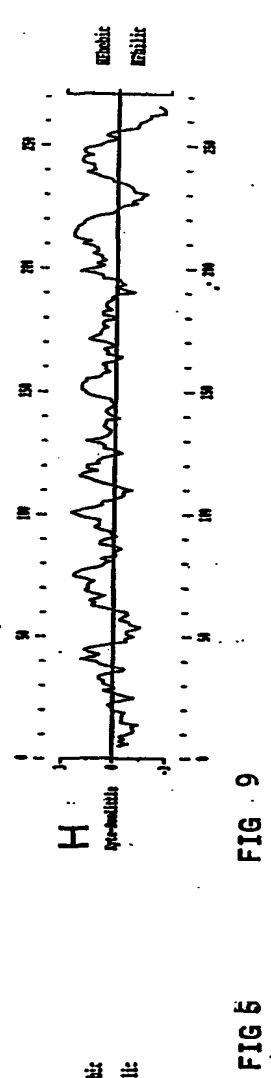
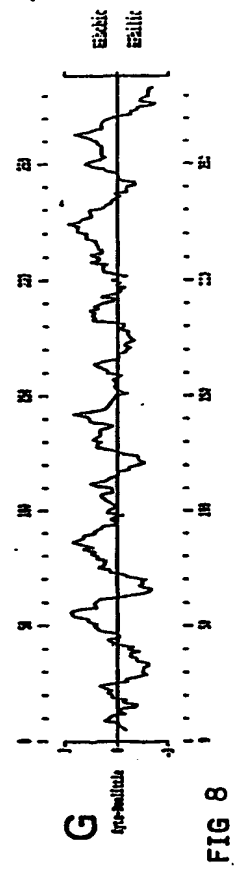
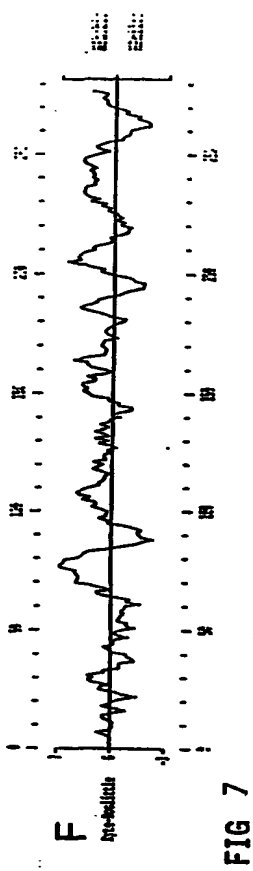
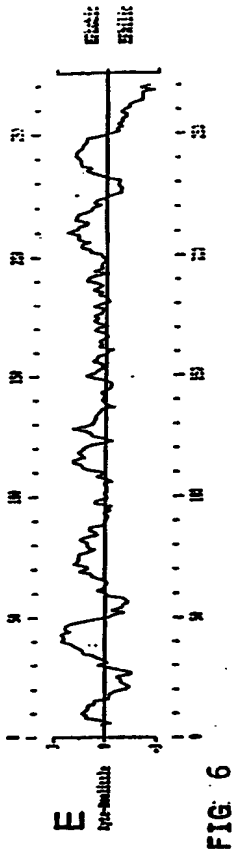


FIG. 10

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Elo3 MNTTTSTVIAAVADQFQSLNSSSSCFLKVHVPSIENP-FGIEWPISFKVFEYFSG--YP
Cig30 -----MDTSMNF8RGLKMDLMO
B207d4 -----
F56H11.4 -----MAQHPLVQRQLDVKFDTKRFVAIATHG
F41H10.8 -----MPQGEVSFFEVLTTA
F56H11.3 -----MYLNYFATEIFHRSAVCETEACRSSKIMIADVFKWKFDANELWSLLTNQ

Elo1 PKDPEFTVGKQPLSEPR--PVLLFIAMTYMIFGGRSLVK--SKPKLKRFLISQVHLM
Elo2 PSEFQFIAGELPLSTLP--PVLYAITAYYIIFGGRFLIS--KSKPKLNGLFQIHLVL
Elo3 AEQFEFIHNKTFLANGY--HAVSITIVYIIFGGQAHLALNASPLKFLFEIHLFL
Cig30 PYDETETFDQLRPFLEEWVSSFLIVVVYLLLVVGSQTYKR--TRKSPSLQRPLILVSSFFL
B207d4 -----
F56H11.4 PKNPPDAEG-RKFFADHFDVTIQASILAVVVFGTQWFR--NRQPFQITIPNINWFI
F41H10.8 P--PSHELK-KKHIAQTQYAAFWISMAVYVIFGLKAVYI--NRKPFDTLQPLNINWAGL
F56H11.3 DEVEPHIR-ARRFIQEHFGLFYQMATAVVILVPSIKRFR--DRPPQITTLALRLWVFFL

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Cig30 AIFSILGTLRAWKFMATVMTVGLKQTVCFIYTDAMVRFWSFLFLSKVVELQDTAFI
B207d4 -----
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F41H10.8 AIFSTLGSATTFGLHEFESRGEFESYIHIGDFYNGISGMRTWFLSKVAEFGDTLFI
F56H11.3 SVFSIYGSWTFFPMVQQLRLYGYGCGCEALSNLPSQAEYWLFTILSKAVEFVDTIFI

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Elo3 VLRRKRLTEFLTYHGGATALLCYTQLIGRTSVEWVVLNLNLGVHVMRYWYFFLSASGIRV
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B207d4 ILRKRPLIFLHWYHSTVLLSYWSYKMDVAGGGWFMILNFGVHAMYSYYALRAACFRV
F56H11.4 VLRKRPLIFLHWYHSTVLLMIYAWYSHPLTPGFNRYGRIYLNFFVHAFMYSYYFLRSMKIRV
F41H10.8 ILRKRPLIFLHWYHSTVLLMNYAFMSFEANLGFNTWITWVNF8VHSIMYGYMLRSFQVKV
F56H11.3 VLRKRPLIFLHWYHSTVLLFVFFCSNYPTSSQSRVGYIYNLFVHAFMYPYETRSMNKKV

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Elo2 --WKQWYTRFOIIGFVLDIGFIYFAVYQKAVHLYF--PIUPHCGDGVG8TTATFAGCAI
Elo3 --WKQWYTRFOIIGFVLDVVFYFATYTFYAHKYL-DGILPNKGTQYGTQAAAAYGYFI
Cig30 PNL LPMVITLSQITQMLMGT-IFG-IF-----NYIW--RQEKGCHTTTEHFW-SFML
B207d4 SRKFAMFITLSQITQMLMGC-VVN-YL-----VFCW--MQHDQCH8HFQNI FW-8SML
F56H11.4 PGI AQAITLSQITQMLSCAVLA-HL-----GYLM--HFTNANCOFEP8VFKLAVFM
F41H10.8 PAKIAKNITTMQITQMLTHFLE-HV-----GYLA--VTGQSDSTPGYWFCLIM
F56H11.3 PAKISMAVITLQITQML-CFIYGCTE-----MYYS--LATNQARYPSNTPATLQCLIS

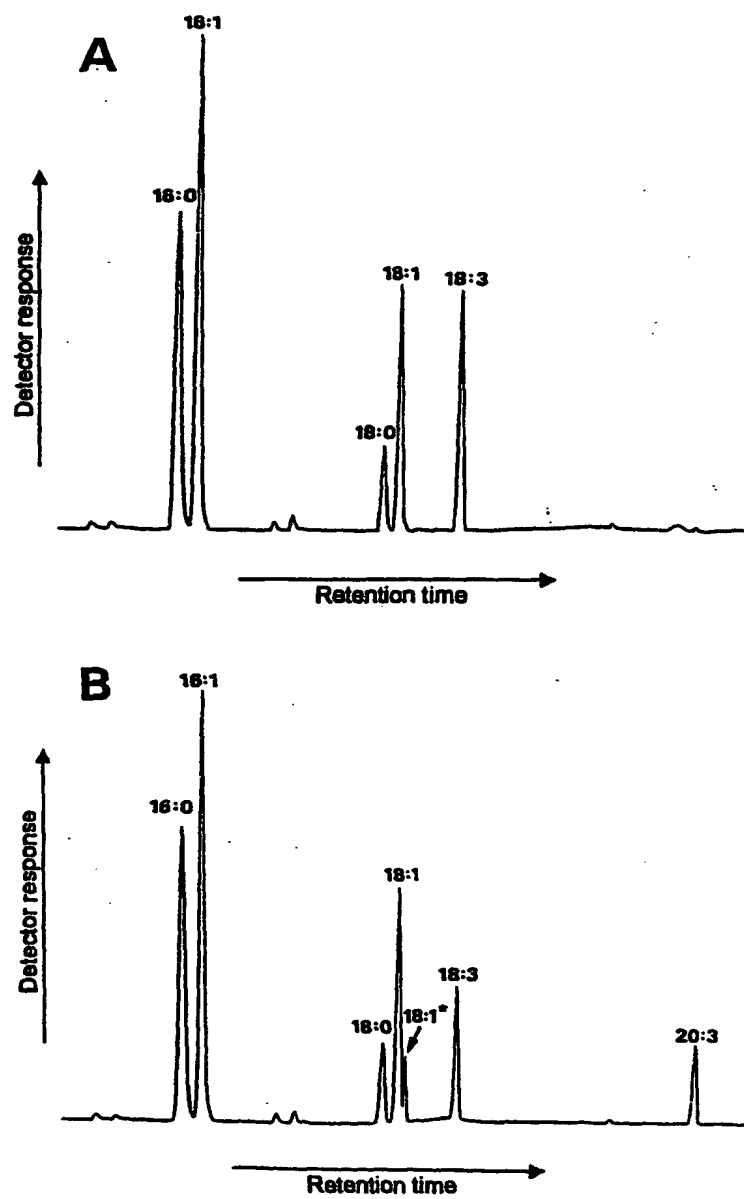
Elo1 LTSYLFLFISFYIEVYKRG8ASGKKIKNN-----
Elo2 ISSYLVLFI8FYINVKRSTSTSRVVKRAHGGVAAKVNEYVNVDLKNVPTSPSPKPKQH
Elo3 LTSYLLFI8FYIQSYKKGSKTVKRESEVSG8VAG8S8TGKTSNTKV88RKA-----
Cig30 YGTIVFLEAHFFHRAVLRPKGVASISQ-----
B207d4 YLSYLVLFCHFFFEAYI-----
F56H11.4 DTITYLALFVNFFLQSYVLRSGDKYKAVPKKKNN-----
F41H10.8 EISYVVLFGNFYQSYIKGSGK-KFNAEKKTEKKIE-----
F56H11.3 YILHL-----

Elo1 -----
Elo2 RRRK
Elo3 -----
Cig30 -----
B207d4 -----
F56H11.4 -----
F41H10.8 -----
F56H11.3 -----

```

4/4

FIG. 11



INTERNATIONAL SEARCH REPORT

International Application No

PC 7/98 00/01035

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/54 C12N9/10 C12N1/21 A01H5/00 C07C57/03
C07C57/12 A61K38/45 A61K31/202 A23L1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE SWALL 'Online! EMBL Heidelberg, Germany; ID: YLF4_CAEEL, AC: Q03574, 1 February 1994 (1994-02-01) WILSON R ET AL.: "2.2 Mb of contiguous nucleotide sequence from chromosome III of C. elegans" XP002143744 abstract --- -/-	1-12, 18, 21, 24

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

28 July 2000

Date of mailing of the international search report

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Authorized officer

Oderwald, H

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMINV 'Online! EMBL Heidelberg, Germany; ID: CEC40H1, AC: Z19154, 27 December 1992 (1992-12-27) BERKS M: "Caenorhabditis elegans cosmid C40H1" XP002143745 see nucleotides 18500 to 20600 abstract</p>	16,17
X	<p>JAMES D W ET AL: "DIRECTED TAGGING OF THE ARABIDOPSIS FATTY ACID ELONGATION1 (FAE1) GENE WITH THE MAIZE TRANSPOREN ACTIVATOR" PLANT CELL,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 7, March 1995 (1995-03), pages 309-319, XP002911493 ISSN: 1040-4651 cited in the application the whole document</p>	1-3,7,8, 16-18, 21-23, 27-29
A	<p>WATTS J L AND BROWNE J: "Isolation and characterization of a delta5-fatty acid desaturase from Caenorhabditis elegans" ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 362, no. 1, 1 February 1999 (1999-02-01), pages 175-182, XP002143742 the whole document</p>	22
A	<p>NAPIER ET AL: "Identification of a Caenorhabditis elegans delta6-fatty-acid-desaturase by heterologous expression in Saccharomyces cerevisiae" BIOCHEMICAL JOURNAL,GB,PORTLAND PRESS, LONDON, vol. 330, no. 2, March 1998 (1998-03), pages 611-614-614, XP002099453 ISSN: 0264-6021 the whole document</p>	23
A	<p>SALEM N ET AL: "Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 93, no. 93, January 1996 (1996-01), pages 49-54-54, XP002131822 ISSN: 0027-8424 the whole document</p>	

-/-

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01035

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OH ET AL: "ELO2 and ELO3, homologs of the Saccharomyces cerevisiae ELO1 gene, function in fatty acid elongation and are required for sphingolipid formation" JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 28, 11 July 1997 (1997-07-11), pages 17376-17384, XP002119019 ISSN: 0021-9258 cited in the application the whole document	
P, X	WO 00 12720 A (ABBOTT LAB) 9 March 2000 (2000-03-09) the whole document	1-3, 7-31, 35

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/01035

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 38-40 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/01035

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0012720 A	09-03-2000	AU 5696499 A	21-03-2000

CORRECTED VERSION

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(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,
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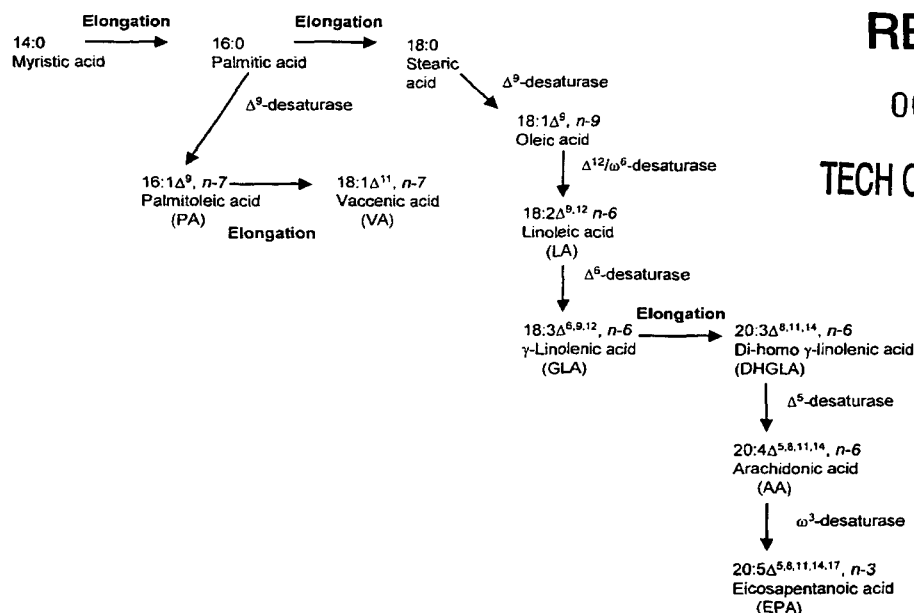
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(54) Title: POLYSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS



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WO 00/055330 A1

(57) Abstract: A isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase.



(15) Information about Correction:

see PCT Gazette No. 35/2002 of 29 August 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

POLYSATURATED FATTY ACID (PUFA) ELONGASE FROM CAENORHABDITIS ELEGANS

The present invention relates to polyunsaturated fatty acid (PUFA) elongases. More specifically, the invention relates to a DNA sequence from *C. elegans* encoding a PUFA elongase.

Unsaturated fatty acids are essential components required for normal cellular function, being involved in a diverse number of roles ranging from membrane fluidity to acting as signal molecules (Gill, I., Valivety, R. (1997). *Trends Biotechnol.* 15, 401-409; Broun, P., *et al* (1999) *Ann. Rev. Nutr.* 19, 197-216). In particular, the class of fatty acids known as the polyunsaturated fatty acids (PUFAs) has attracted considerable interest as pharmaceutical and nutraceutical compounds (Broun *supra*; Horrobin, D. F. (1990) *Reviews in Contemp Pharmacotherapy* 1, 1-45).

The synthesis of PUFAs i.e. fatty acids of 18 carbons or more in length and containing two or more double bonds, is thought to be catalyzed in a variety of organisms by a specific fatty acid elongase enzyme. This elongase is responsible for the addition of 2 carbon units to an 18 carbon PUFA, resulting in a 20 carbon fatty acid. An example of this reaction is the elongation of γ -linolenic acid (GLA; 18:3 $\Delta^{6,9,12}$) to di-homo- γ -linolenic acid (DHGLA; 20:3 $\Delta^{8,11,14}$) in which the tri-unsaturated 18 carbon fatty acid is elongated by the addition of a two carbon unit to yield the tri-unsaturated 20 carbon fatty acid. Since there is considerable interest in the production of long chain PUFAs of more than 18 carbons in chain length, for example arachidonic acid and eicosapentanoic acid, the identification of this enzyme is of both academic and commercial interest.

At present, there are no examples of identified cloned genes encoding PUFA elongases, though a number of genes encoding enzymes likely to be involved in other aspects of lipid synthesis have been identified. For example, an *Arabidopsis* gene (FAE1) has been shown to be required for the synthesis of very long chain monounsaturated fatty acids (such as erucic acid; 20:1 Δ^{11}) (James, D. W. *et al*, (1995) *Plant Cell* 7, 309-319). However, it is clear that this enzyme does not recognize di- and tri-unsaturated 18 carbon fatty acids, for example, linoleic acid, 18:2 $\Delta^{9,12}$ or α -linolenic acid, 18:3 $\Delta^{9,12,15}$ respectively, as substrates,

and is therefore not involved in the synthesis of long chain PUFAs (Millar & Kunst (1997), *Plant Journal* **12**, 121-131). This in itself is not surprising, since, of the plant kingdom, only a very few lower plant species, such as the moss *Physcomitrella patens* (Girke *et al.*, (1998), *Plant J*, **15**: 39-48); are capable of synthesising long chain PUFAs, and therefore *Arabidopsis* would not be expected to contain any such enzymes (Napier *et al.* (1997), *Biochem J*, **328**: 717-720; Napier *et al.*, (1999) *Trends in Plant Sci* **4**, 2-5).

A schematic diagram representing a generalized pathway for the product of PUFAs is shown in Figure 1. Biochemical characterisation of mammalian elongation systems (most notably from liver microsomes) has indicated that a mammalian elongase consists of four subunits, made up of a condensing enzyme, a β -ketoreductase, a dehydrase and an enoyl reductase (reviewed in Cinti, D. L., *et al* (1992) *Prog. Lipid Res.* **31**, 1-51). The *Arabidopsis FAE1* gene product encodes a polypeptide of 56kDa, which shows very limited homology to condensing enzymes such as chalcone synthase and stilbene synthase (James, D. W. *supra*). Although *FAE1* is normally only expressed in seed tissues, ectopic expression in non-seed tissue (or heterologously in yeast) revealed that *FAE1* could direct the synthesis of erucic acid (Millar, A. A., Kunst, L. (1997) *Plant J.* **12**, 121-131).

Three fatty acid elongase activities have been characterised from the yeast *S. cerevisiae*. Again, this organism does not synthesise PUFAs, and therefore does not contain genes encoding a PUFA elongase. One gene *ELO1*, was identified on the basis of a screen to isolate mutants defective in elongation of 14 carbon (i.e. medium) chain saturated fatty acids (Toke & Martin (1996) *J Biol Chem* **271**, 18413-18422). Complementation of *elo1* mutants restored viability, and the *ELO1* gene product was shown to encode a polypeptide which was responsible for the specific elongation of 14:0 fatty acids to 16:0 fatty acids.

Two related genes were also detected in the genome of *S. cerevisiae*, and their function determined by disruption. These two genes, subsequently named *ELO2* and *ELO3*, were shown to be involved in the elongation of the very long chain saturated fatty acids found in sphingolipid molecules (Oh *et al* (1997), *J. Biol Chem* **272**, 17376-17384). In particular, *ELO2* was required for elongation of fatty acids up to 24 carbons, and *ELO3* was required for elongation of the 24 carbon fatty acid to 26 carbons. However, neither gene was

essential for viability. Examination of the these three fatty acid elongases revealed the presence of a conserved "histidine box" motif (Shanklin *et al.*, (1994), *Biochemistry*, 33, 12787-12794) (His-X-X-His-His, where X is any amino acid) towards the centre of the polypeptide sequences. Importantly, there was no detectable homology between the yeast elongases (ELO1,2,3) and the plant very long chain mono-unsaturated fatty acid elongase (FAE1) (Oh *et al*, *supra*).

In order to identify genes encoding PUFA elongases, it is necessary to study systems in which the synthesis of PUFAs is well documented; a good example of this is the model animal system *C. elegans*, a small free-living worm (Tanaka *et al.*, (1996), *Lipids* 31, 1173-1178). *C. elegans*, like most other animals, and in contrast to higher plants, synthesises PUFAs such as arachidonic acid (AA; 20:4 $\Delta^{5,8,11,14}$) as precursors to a class of molecules known as the eicosanoids, which in turn serve as precursors for compounds such as prostaglandins and leucotrienes (Horrobin, (1990), *Reviews in Contemp Pharmacotherapy*, 1:1-45). The presence of AA and other long chain polyunsaturated fatty acids in *C. elegans* is well documented (Tanaka *et al.*, (1996), *Lipids* 31, 1173-1178). The complete sequence of the nematode's genome is now publicly available (*The C. elegans consortium*, 1998, *Science* 282, 2012-2018: Database at http://www.sanger.ac.uk/Projects/C_elgans/blast_server.shtml).

An object of the invention is to provide an isolated PUFA elongase.

Using the above-mentioned *C. elegans* genomic sequence, together with suitable search strings, the inventors identified eight related putative open reading frames (ORFs) encoding for PUFA elongases. A number of different search criteria were applied to identify a number of (ORFs) which were likely to encode polypeptides with fatty acid elongase activities. These ORFs were then subject to functional characterisation by heterologous expression in yeast, allowing the identification of a PUFA elongase.

Accordingly, a first aspect of the invention provides an isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase i.e. the polypeptide has the function of extending the chain length of an 18 carbon PUFA to 20 carbons in length.

This polypeptide can be used to elevate PUFA levels in animals, thereby providing a ready source of PUFAs.

The polypeptide may be from a eukaryote.

The polypeptide may comprise at least a portion of the amino acid shown in SEQ ID. 15, or variants thereof.

For the purposes of the present application, the term "variant" in relation to a certain sequence means a protein or polypeptide which is derived from the sequence through the insertion or deletion of one or more amino acid residues or the substitution of one or more amino acid residues with amino acid residues having similar properties, e.g. the replacement of a polar amino acid residue with another polar amino acid residue, or the replacement of a non-polar amino acid residue with another non-polar amino acid residue. In all cases, variants must have an elongase function as defined herein.

A second aspect of the invention provides a polypeptide having at least 60 % homology to a polypeptide according to a first aspect of the invention. The polypeptide may have at least 80%, or as much as 90% or more homology to a polypeptide according to a first aspect of the invention.

The polypeptide according to either aspect of the invention may include a sequence motif responsible for Endoplasmic Reticulum (ER) - retention. This allows the polypeptide to be specifically located or targeted to the ER of a cell.

The polypeptide may also be able to elongate palmitoleic acid (PA; 16:1 Δ^9) to vaccenic acid (VA; 18:1 Δ^{11}). Thus, the polypeptide is also capable of elongation of a Δ^9 - monounsaturated 16C fatty acid.

Preferably, the polypeptide is from an animal, more preferably, the animal is an invertebrate such as a worm. Where the animal is a worm, it is preferably *C. elegans*. Alternatively, the animal is a vertebrate, preferably a mammal such as a human, rat or mouse.

A third aspect of the invention provides an isolated DNA sequence, preferably a cDNA sequence, encoding a polypeptide according to a first or second aspect of the invention. This DNA sequence may be used to engineer transgenic organisms.

Preferably, the DNA sequence comprises the sequence shown in SEQ ID NO: 7 or variants of that sequence due, for example, to base substitutions, deletions, and/or additions.

A fourth aspect of the invention provides an engineered organism, such as a transgenic animal, engineered to express a polypeptide according to a first or second aspect of the invention. The engineered organism may be engineered to express elevated levels of the polypeptide, thereby providing a supply of polypeptide at a reduced cost as a reduced number of organisms need be used.

Preferably, the engineered organism is a mammal such as a rat, mouse or monkey.

A fifth aspect of the invention provides an engineered organism containing a synthetic pathway for the production of a polypeptide according to a first or second aspect of the invention. This has the advantage of allowing greater control over the production of PUFAs by the pathway by an organism.

The pathway may include Δ^5 -fatty acid desaturase, and/or Δ^6 -fatty acid desaturase.

The engineered organism according to a fourth or fifth aspect of the invention may be a lower eukaryote, such as yeast. Alternatively, the transgenic organism may be a fish.

A sixth aspect of the invention provides a transgenic plant engineered to express a polypeptide according to a first aspect of the invention.

A seventh aspect of the invention provides a transgenic plant containing a DNA sequence according to a third aspect of the invention.

An eighth aspect of the invention provides a method of producing a PUFA comprising carrying out an elongase reaction catalysed by a polypeptide according to a first or second aspect of the invention.

The PUFA may be di-homo-gamma-linoleic acid ($20:3\Delta^{8,11,14}$), arachidonic acid ($20:4\Delta^{5,8,11,14}$), eicosapentanoic acid ($20:5\Delta^{5,8,11,14,17}$), docosatrienoic acid ($22:3\Delta^{3,16,19}$), docosatetraenoic acid ($22:4\Delta^{7,10,13,16}$), docosapentaenoic acid ($22:5\Delta^{7,10,13,16,19}$) or docosahexaenoic acid ($22:6\Delta^{4,7,10,13,16,19}$).

The PUFA may be a 24 carbon fatty acid with at least 4 double bonds.

A ninth aspect of the invention provides a PUFA produced by a method according to an eighth aspect of the invention.

The PUFA may be used in foodstuffs, dietary supplements or pharmaceutical compositions.

A tenth aspect of the invention provides a foodstuff comprising a PUFA according to a fifth aspect of the invention. The foodstuff can be fed to an animal.

An eleventh aspect of the invention provides a dietary supplement comprising a PUFA according to a fifth aspect of the invention. The dietary supplement can be supplied to an animal to augment its PUFA levels.

An twelfth aspect of the invention provides a pharmaceutical composition comprising a polypeptide according to a first or second aspect of the invention or a PUFA according to a ninth aspect of the invention.

Preferably, the pharmaceutical composition comprises a pharmaceutically-acceptable diluent, carrier, excipient or extender. This allows the composition to be supplied in a form which best suits the pharmaceutical application in question. For example, a topical application would preferably be a cream or lotion, whereas if the composition was to be ingested a different form would be more suitable.

A thirteenth aspect of the invention provides a method of treatment of an animal, such as a mammal, or a plant, comprising supplying to the animal or plant a DNA sequence according to a third aspect of the invention, a foodstuff according to a tenth aspect of the invention, a dietary supplement according to an eleventh aspect of the invention, a pharmaceutical composition according to a twelfth aspect of the invention or a PUFA according to a ninth aspect of the invention.

Preferably, the mammal is a human.

The invention will now be further described, by way of example only, with reference to SEQ ID1 to 16, and Figures 2 to 11, in which;

SEQ ID1 to 8 show the putative ORFs encoding PUFA elongases A to H respectively; and

SEQ ID9 to 16 show the deduced amino acid sequences of the putative ORFs of SEQ ID NO: 1 to 8 respectively; and

Figures 2 to 9 show hydrophobicity plots for each of PUFA elongases A to H respectively.

Figure 10 shows an amino acid sequence line-up comparing the *C. elegans* ORF F56H11.4 (Z68749) with related sequences.

Figure 11 shows chromatograms of fatty acid methyl esters from transformed yeast.

Introduction to general strategy

Initially the *C. elegans* databases were searched for any sequences which showed low levels of homology to yeast ELO genes (*ELO2* and *ELO3*) using the TBLASTN programme. A similar search was carried out using short (20 to 50 amino acid) stretches of ELO genes which were conserved amongst the three ELO polypeptide sequences. *C. elegans* sequences which were identified by this method were then used themselves as search probes, to identify any related *C. elegans* genes which the initial search with the yeast sequences failed to identify. This was necessary because the level of homology between the yeast ELO genes

and any worm genes is always low (see BLAST scores later). To allow for a more sensitive search of worm sequences, a novel approach was adopted to circumvent the major drawback with searches using the BLAST programmes, namely that the search string (i.e. the input search motif) must be longer than 15 characters for the algorithm to work. Thus, if it was desired to search for a short motif (like a histidine box), then the BLAST programme would not be capable of doing this. A complete list of all the predicted ORFs present in the *C. elegans* genome exists as a database called Wormpep, which is freely available from the Sanger WWW site (http://www.sanger.ac.uk/Projects/C_elegans/webace_front_end.shtml). The latest version of Wormpep was down loaded to the hard disc of a Pentium PC, and re-formatted as a Microsoft Word6 document, resulting in a document of about 3,500 pages. This was then searched using the "Search & Replace" function of Word6, which also allows for the introduction of "wildcard" characters into the search motif. So, for example, it is possible to search both for the short text string HPGG, which would identify any predicted worm ORF present in the Wormpep 3,500 page document containing this motif, or alternatively search with HPGX (where X is a wild card character). Clearly, such (manual) searches of a 3,500 page document are extremely time-consuming and demanding, also requiring visual inspection of each and every identified ORF. For example, searching with a motif such as HXXHH identifies in excess of 300 different ORFs. However, by using a number of different short search strings (as outlined below), and combining these with other methods for identifying putative elongase enzymes, a number of candidate ORFs have been identified.

Database search using the FAE1 polypeptide sequence

As a negative control, to demonstrate that the FAE1 gene sequence was unlikely to provide a useful search sequence in the identification of *C.elegans* sequences encoding for PUFA elongases, the GenBank databases (<http://www.ncbi.nlm.nih.gov/Web/Search/index.html>) were searched using the *Arabidopsis* FAE1 polypeptide sequence to identify related genes or expressed sequence transcripts (ESTs). GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (*Nucleic Acid Research* (1998) **26**, 1-7). There are approximately 2,162,000,000 bases in 3,044,000 sequence records as of December 1998. The search was carried out using the BLAST2 (Basic Local Alignment Search Tool) algorithm (Altschul *et al.*, (1990) *J Mol Biol* **215**,403,410) Although a number

of plant ORFs and ESTs were reported as being related, no animal sequences were identified by this search, confirming the observation that FAE1 was unlikely to be a suitable candidate as a search template for PUFA elongases.

Database search using yeast ELO sequences

Using the three yeast fatty acid elongase sequences (ELO 1, 2, 3) as probes, a number of putative ORFs in the DNA of *C. elegans*-derived cosmid sequences which form the *C. elegans* genomic sequence database were identified. Moreover, an extensive and time-consuming search of a downloaded copy of the WormPep database (<ftp://ftp.sanger.ac.uk/pub/databases/wormpep>) using manual search strings in MSWord 6, identified a number of *C. elegans* ORFs which contained presumptive histidine boxes. Wormpep contains predicted proteins from the *Caenorhabditis elegans* genome sequence project, which is carried out jointly by the Sanger Centre in Cambridge, UK and Genome Sequencing Center in St. Louis, USA. The current Wormpep database, Wormpep 16, contains 16,332 protein sequences (7,120,115 residues). Search strings used included [HXXHH], [HXXXHH], [QXXHH] and [YHH]. Comparison of the data from the two different searches indicated a small (<10) number of putative ORFs as candidate elongases. The histidine box motifs are shown in bold in SEQ ID 9 to 16.

Hydrophobicity plot analysis

Since the fatty acid elongase reaction is predicted to be carried out on the cytosolic face of the endomembrane system (Toke & Martin (1996), *supra*; Oh *et al* (1997), *supra*), the putative *C. elegans* ORFs were examined for potential membrane spanning domains, via Kyte & Doolittle hydrophobicity plots (*J. Mol Biol*, (1982), **157**, 105-132). This revealed a number of ORFs with possible membrane-spanning domains, and also indicated a degree of similarity in the secondary-structure of a number of identified ORFs.

Screening for ER-retention signal sequences

The inventors postulated that since fatty acid elongases are expected to be endoplasmic reticulum (ER) membrane proteins, they might be expected to have peptide signals which are responsible for "ER-retention". In the case of ER membrane proteins, this signal often takes the form of a C-terminal motif [K-K-X₂₋₃-Stop], or similar variants thereof (Jackson *et*

al., (1990), *EMBO J.*, **9**, 3153-3162). Further sequence analysis of the *C. elegans* putative elongases revealed that 4 ORFs (F41H10.7, F41H10.8, F56H11.4, Y53F4B.c) had C-terminal motifs that exactly matched this search pattern, and that a further 2 ORFs (F11E6.5, C40H1.4) had related sequences. These sequence motifs are underlined in SEQ ID 9 to 13, 15 and 16.

Chromosome mapping

Since the inventors had previously observed that *C. elegans* genes involved in the synthesis of PUFA may exist in tandem (for example the $\Delta 5$ and $\Delta 6$ desaturases required for AA and GLA synthesis, respectively, are < 1 kB apart on chromosome IV (Michaelson *et al.*, (1998), *FEBS Letts* **439**, 215-218), the positions of the putative *C. elegans* elongase ORFs were determined using the Sanger Centre's WebAce *C. elegans* server (http://www.sanger.ac.uk/Projects/C_elegans/webace_front_end.shtml). This indicated that two pairs of putative elongases were in close proximity to each other on the *C. elegans* chromosome IV.

F41H10.7 and F41H10.8 were identified as being approximately 10 Kb apart on chromosome IV, and F56H11.3 and F56H11.4 were identified as being approximately 2 Kb apart on chromosome IV.

Putative *C. elegans* fatty acid elongases

The positions of the putative ORFs in the *C. elegans* genome are shown below i.e. chromosome number, and map position in centiMorgans, together with the GenBank database accession numbers.

The designations used employ the same method as used on the Sanger Centre's *C. elegans* database, i.e. ORF C40H1.4 is predicted coding sequence 4 on cosmid C40H1.

<u>Elongase</u>	<u>Cosmid Sanger ID</u> <u>Code</u>	<u>GenBank Acc</u>	<u>Chromosome</u>
A	C40H1.4	Z19154	III

B	D2024.3	U41011	IV, 7.68
C	F11E6.5	Z81058	IV, 18.8
D	F41H10.7*	U61954	IV, 29.8
E	F41H10.8*	U61954	IV, 29.8
F	F56H11.3 [#]	Z68749	IV, 2.5
G	F56H11.4 [#]	Z68749	IV, 2.5
H	Y53F4B.c	Z92860	II

* or[#] indicates genes in tandem

Comparison of *C. elegans* putative elongase ORFs with yeast genes:

Each of the three yeast ELO polypeptides were compared against all of the worm putative elongase translated ORF sequences, and then ranked in order of similarity (as measured by the BLAST score) (Altschul *et al* (1990), *supra*)

The results are shown below, with the ORF sequences ranked from most similar to least similar, and the BLAST scores are shown in brackets:

Yeast ELO1 (14 to 16 carbon fatty acid elongase)

G (262) > E (241) > D (225) > C (219) > A (216) > F (215) > H (197) > B (172)

Yeast ELO2 (24 carbon sphingolipid elongase)

E (231) > C (226) > G (189) > A (181) > F (166) > D (150) > H (141) > B (140)

Yeast ELO3 (24 to 26 sphingolipid elongase)

D (171) > G (163) > F (154) > A (152) > E (150) > C (131) > B (132) > H (128)

It is clear from the numeric values of the BLAST scores that the sequences are related, but the levels of homology are low. For comparison, the BLAST score for homology between two related worm proteins, the $\Delta 5$ and the $\Delta 6$ desaturase is in excess of 500.

Analysis of potential sphingolipid ancestry

Previously, the inventors had noted the similarities between the fatty acid $\Delta 6$ desaturase and sphingolipid desaturases in plants, and that the two distinct enzymes could have arisen from one ancestral gene. Moreover, it was considered likely that the sphingolipid desaturase predated the fatty acid desaturase, and may in fact have been the ancestral progenitor. Therefore it is plausible that the next step in the arachidonic acid biosynthetic pathway has also evolved from the sphingolipid metabolic pathway. It is therefore considered highly significant that some of the *C. elegans* ORF putative elongases have similarity to sphingolipid enzymes. For this reason, these ORFs are considered to be very clear candidates for PUFA elongases. It has previously been considered that the *C. elegans* $\Delta 5$ and $\Delta 6$ fatty acid desaturases have evolved from 1 ancestral gene (Michaelson *et al.*, (1998), *FEBS Letts* 439, 215-218). It is also significant that one pair of *C. elegans* putative elongase ORFs (F & G) genetically maps close to the $\Delta 5/\Delta 6$ fatty acid desaturase genes, with both gene pairs being located at the top end of chromosome IV.

<u>Cosmid Sanger ID Code</u>	<u>GenBank Acc</u>	<u>Chromosome</u>	<u>Encoded Peptide</u>
W08D2.4	Z70271	IV, 3.06	$\Delta 6$ fatty acid desaturase
T13F2.1	Z81122	IV, 3.06	$\Delta 5$ fatty acid desaturase

Cloning of Desaturase and Elongase Genes in Yeast Expression Vectors

Putative elongases sequences F56H11.4 and F41H10.8 were cloned by PCR into the pYES2 vector (Invitrogen). A *C. elegans* mixed stage cDNA library was used as a PCR template. F56H11.4 was amplified using primers:

56h114.for 5'-GCGGGTACCATGGCTCAGCATCCGCTC-3' and;

56h114.rev 5'-GCGGGATCCTTAGTTGTTCTTCTTCTT-3'.

F41H10.8 was amplified using primers:

41h108.for 5'-GCGGGTACCATGCCACAGGGAGAAGTC-3' and;

41h108.rev 5'-GCGGGATCCTTATTCAATTTTCTTTT-3'.

Amplified sequences were then restricted using *KpnI* and *BamHI* (underlined in the forward and reverse primers, respectively), purified using the Qiagen PCR purification kit, and ligated into a *KpnI/BamHI* cut pYes2 vector.

An ORF encoding the *Mortierella alpina* Δ^5 -fatty acid desaturase (Michaelson, L. V., *et al* (1998) *J. Biol. Chem.* **273**, 19055-19059) was amplified using primers:

Mad5.for 5'-GCGAATTCACCATGGGTACGGACCAAGGA-3' and;

Mad5.rev 5'-GCGGAGCTCCTACTCTTCCTTGGGACG-3',

and restricted using *EcoRI* and *SacI*, gel purified as described and ligated into a *EcoRI/SacI* cut pESC-TRP vector (Stratagene) to generate pESC/ Δ^5 .

An ORF encoding the borage Δ^6 -fatty acid desaturase (Sayanova, O., *et al* (1997) *Proc. Natl. Acad. Sci USA* **94**, 4211-4216) was restricted from pGEM3 using *BamHI* and *XhoI* and ligated into a *BamHI/XhoI* cut pESC-TRP vector to generate pESC/ Δ^6 .

A double construct was also generated by ligating the *Bam*HI/*Xho*I borage Δ^6 insert into the pESC/ Δ^5 construct described previously, generating pESC/(Δ^5, Δ^6).

Functional Characterisation in Yeast

Elongases and desaturase constructs were introduced in *Saccharomyces cerevisiae* W303-1A using a lithium acetate based method (Elble, R. (1992) *Biotechniques* 13, 18-20) and expression of the transgenes was induced by addition of galactose to 2% (w/v) as described in Napier *et al* (Napier, J. A., *et al* (1998) *Biochem J* 330, 611-614; Michaelson L. V., *supra*; Michaelson, L. V., (1998) *FEBS Letts* 439, 215-218). Yeast transformants containing pYES2-derived constructs were grown on synthetic minimal media (SD, the composition of which is defined in Sherman, F (1991) *Methods in Enzymology* 194, 3-21); synthetic minimal medium minus uracil; pESC-derived constructs were grown on SD minimal medium minus tryptophan. Co-transformed yeast (containing both pYES2 and pESC derivatives) were grown on SD minimal medium minus uracil and tryptophan. Prior to induction, cultures were grown in the presence of 2% raffinose and supplemented with 0.5 mM of the appropriate fatty acid substrate in the presence of 1% tergitol-(NP40) (Sigma). All cultures were then grown for a further 48-h unless indicated.

Fatty Acid Analysis

To identify the elongation reaction responsible for the synthesis of di-homo- γ -linolenic acid (DHGLA; 20:3 $\Delta^{8,11,14}$) from GLA, this latter fatty acid was supplied as the (exogenous) substrate.

Lipids were extracted from transformed and control yeast by homogenisation in MeOH-CHCl₃ using a modification of the method of Bligh and Dyer (Dickenson & Lester (1999) *Biochim Biophys Acta* 1426, 347-357). The resulting CHCl₃ phase was evaporated to dryness under nitrogen gas and the samples were transmethylated with 1M HCl in methanol at 80 °C for 1 hour. Fatty acid methyl esters (FAMES) were extracted in hexane and purified using a small column packed with Florisil. Analysis of FAMES was conducted using a Hewlett Packard 5880A Series Gas Chromatograph equipped with a 25M x 0.32mm RSL-500BP bonded capillary column and a flame ionisation detector. Fatty acids were identified by comparison of retention times with FAME standards (Sigma)

separated on the same GC. Quantitation was carried out using peak height area integrals expressed as a total of all integrals (Bligh, E.G. & Dyer, W.J. (1959) *Can. J. Biochem. Physiol.* 37, 911-917).

Total fatty acids extracted from yeast cultures were analysed by gas chromatography (GC) of methyl ester derivatives. Lipids were extracted, transmethylated and the fatty acid methyl esters (FAMES) analysed as described by Sayanova *et al.*

Figure 11 shows chromatograms of fatty acid methyl esters from yeast transformed with the control (empty) plasmid pYES2 (Fig. 11A) or with ORF F56H11.4 in pYES2 (Fig. 11B). Exogenous substrate in the form of GLA was supplied to the cultures. Two novel peaks are observed in (B); these peaks (annotated as 20:3 and 18:1*) were identified (against known standards) as DHGLA and vaccenic acid, respectively. Detection was by flame ionisation.

One cDNA ORF tested in this manner displayed a high level of elongase activity on the GLA substrate, converting 44% to DHGLA. The identity of this elongation product was confirmed as DHGLA by comparison with a known standard (the standards used were known standards for either DHGLA, AA, EPA or VA from Sigma Chemicals, Ltd.), using GCMS analysis using a Kratos MS80RFA (Napier, J. A., *supra*; Michaelson, L. V., *supra*; Michaelson, L. V., *supra*). The deduced amino acid sequence of the functional elongase clone identified it as being encoded by the *C. elegans* gene F56H11.4, and comparison with the yeast *ELO* genes showed low homology confined to a few short amino acid motifs (see Fig. 10). Some similarity with a mouse gene Cig30 (Tvrdik, P., (1997) *J. Biol. Chem.* 272, 31738-31746), which has been implicated in the recruitment of brown adipose tissue in liver tissue, was also observed, as well as a potential human homologue encoded by a gene located on chromosome 4q25, BAC 207d4. The most closely related *C. elegans* ORFs, F41H10.8 (U61954) and F56H11.3 (Z68749) are also shown, as is part of a related human gene present on chromosome IV (present on BAC clone B207d4; AC004050). The GenBank accession numbers are given for all sequences.

The range of fatty acids synthesised by *C. elegans* can potentially require a number of different elongation reactions (Tanaka, T., (1996) *Lipids* 31, 1173-1178). The substrate-specificity of the F56H11.4 PUFA elongase was therefore determined using a

range of exogenously supplied fatty acids. This revealed that GLA is the major substrate, with a number of other fatty acids being elongated at a lower efficiency (see Table 1). Although most of these substrates are polyunsaturated fatty acids, it was unexpectedly observed that palmitoleic acid (PA; 16:1 Δ^9) was also elongated by F56H11.4 to yield vaccenic acid (VA; 18:1 Δ^{11}). The biosynthetic pathway for VA is unclear, but the data indicate that it may be synthesised by elongation of Δ^9 -monounsaturated 16C fatty acid.

The *C. elegans* PUFA elongase ORF F56H11.4 maps to the top of chromosome IV (at 4.32 cM) with a related sequence (F56H11.3; 51 % similarity) located 1,824bp downstream. Another *C. elegans* gene (F41H10.8) was also observed, which is present on chromosome IV, and which shows a slightly higher level (53%) of similarity to the PUFA elongase than F56H11.3 (see Fig. 10). However, when a PCR product encoding ORF F41H10.8 was expressed in yeast in a manner identical to that used for F56H11.4, the former failed to direct the elongation of any fatty acids, despite the provision of a range of substrates (see Table II).

In order to reconstitute the PUFA biosynthetic pathway in a heterologous system, the PUFA elongase F56H11.4 was expressed in yeast in conjunction with either the Δ^6 - or Δ^5 -fatty acid desaturases previously isolated and characterised by the inventor (Napier, J. A., *supra*; Michaelson, L. V., *supra*). Expression of the Δ^6 -fatty acid desaturase and F56H11.4 was carried out in the presence of two different substrates (LA or ALA) while the Δ^5 -fatty acid desaturase and the elongase were expressed in the presence of GLA only. This demonstrated that was possible to combine a desaturase and an elongase in yeast to generate significant amounts of a final "product" (see Table III). In the case of the elongase and the Δ^6 -fatty acid desaturase, the reactions proved highly efficient with the production of 4.5% of DHGLA from the LA substrate. This resulted from 25% desaturation of the LA substrate to GLA, which was then elongated to DHGLA at a similar level of efficiency (18%). This is lower than the % conversion observed for GLA when supplied exogenously (see Table I), indicating that the *in vivo* production of substrates for elongation may be rate-limiting.

If ALA was used as a substrate, 27% of this was initially Δ^6 -desaturated to yield octadecatetraenoic acid (OTA; 18:4 $\Delta^{6,9,12,15}$) but only 8% of was subsequently elongated to yield eicosatetraenoic acid (20:4 $\Delta^{8,11,14,17}$). Thus, the conversion efficiency of ALA to the final 20-carbon tetraenoic PUFA was only about 2.2%.

Since DHGLA is an *n*-6 fatty acid, whilst the OTA-derived eicostetraenoic acid is an *n*-3 type, this demonstrates that the elongase is capable of accepting both forms of essential fatty acid, albeit with different efficiencies. Verification was also provided that the 20C PUFAs synthesised in the yeast expression system were generated by the Δ^6 -desaturation of 18C substrates which were subsequently elongated, as the Δ^6 -desaturase showed no activity on 20:2 or 20:3 substrates (see Table III).

The combination of the Δ^5 -desaturase and the elongase also demonstrated that these two enzymes could work in tandem, although the efficiency of this overall conversion was lower (3.3% AA from GLA) which was due to the previously observed low activity of the Δ^5 -desaturase enzyme itself (Michaelson, L. V., *supra*; Michaelson, L. V., *supra*). Thus, although nearly 45% of the GLA substrate was elongated to DHGLA, only 7.5% of this was then desaturated to AA (see Table III).

Finally, the production of either AA or eicosapentanoic acid (EPA; 20:5 $\Delta^{5,8,11,14,17}$) in yeast from dienoic or trienoic 18 carbon substrates was achieved via expression of all three enzymes (the two desaturases and the F56H11.4 PUFA elongase) simultaneously. As shown in Table IV, small but significant amounts of AA were produced when the yeast was supplied with the 18C dienoic fatty acid LA.

GC-Mass Spectroscopy (MS) Analysis

Peak identification and confirmation were carried out by GC-MS using a Kratos MS80RFA using known standards (Sigma). The identity of this 20C PUFA was verified by GCMS, indicating that the conversion efficiency from LA was 0.65%. When ALA was used as a substrate, 12.5% of the (Δ^6 -desaturated and elongated) eicosatetraenoic *n*-3 fatty acid was Δ^5 -desaturated, resulting in a total conversion of 0.3% of the ALA substrate to EPA (the identity of EPA was confirmed by GCMS).

Expression of *C. elegans* elongase in plants

In order to express *C. elegans* elongase in plants, the following protocol is an example of a process which can be used to create the transgenic plants. *C. elegans* ORF sequence can be subcloned into a plant expression vector pJD330, which comprises a viral 35S promoter, and a Nos terminator. The resulting cassette or promoter/coding sequence/terminator can then be subcloned into the plant binary transformation vector pBin 19, and the resulting plasmid introduced into *Agrobacterium tumefaciens*. This *Agrobacterium* strain can then be used to transform Arabidopsis by the vacuum-infiltration of inflorescences, and the seeds harvested and plated onto selective media containing kanamycin. Since pBin 19 confers resistance to this antibiotic, only transformed plant material will grow. Resistant lines can therefore be identified and self-fertilized to produce homozygous material. Leaf material can then be analyzed for expression of *C. elegans* elongase.

Fatty acid methyl ester analysis can be carried out as previously described.

Table III

Construct	mole% Fatty Acids						
	Δ^6		$F56H11.4 + \Delta^6$				$F56H11.4 + \Delta^5$
	20:2	20:3	LA		ALA		
Substrate							GLA
Induction	+	-	+	-	+	-	+
16:0	24.7±1.3	25.2±1.5	18.7±0.6	23.7±0.5	17.4±0.7	21.0±1.3	27.9±4.2
16:1	46.0±2.8	43.7±3.7	18.9±1.2	24.6±0.7	5.3±0.6	9.1±0.9	24.6±3.4
16:2	5.2±1.2	4.1±1.4	0.6±0.1	-	0.4±0.1	-	-
18:0	4.8±0.4	5.1±0.4	4.0±0.3	5.1±0.1	6.2±0.7	5.4±0.2	5.6±0.8
18:1	15.3±1.1	16.1±1.2	12.2±1.4	11.2±0.4	5.7±0.8	6.0±0.4	12.7±2.9
18:1*	-	-	7.7±0.7	-	2.6±0.3	-	2.9±0.9
LA	-	-	25.0±3.2	35.4±2.1	-	-	-
ALA	-	-	-	-	42.3±3.3	58.5±4.7	-
GLA	-	-	7.9±2.2	-	-	-	13.2±3.6
OTA	-	-	-	-	15.3±1.8	-	-
20:2	4.0±0.3	-	3.3±0.5	-	-	-	-
DHGLA	-	-	1.7±0.2	-	-	-	-
20:3	-	5.8±0.5	-	-	3.4±0.4	-	-
AA	-	-	-	-	-	-	-
20:4	-	-	-	-	1.4±0.2	-	-
EPA	-	-	-	-	-	-	-
% Elongated							
GLA	-	-	17.7	-	-	-	44.5
OTA	-	-	-	-	8.4	-	-
LA	-	-	8.7	-	-	-	-
ALA	-	-	-	-	5.4	-	-

SEQ ID1

C40H1.4

atggagcttgccgagttctggaatgatctcaacaccttcaccatctacggaccgaatcac
acagatatgaccacaaaatacaaatattcatatcacttcccaggtgaacaggtggcgat
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SEQ ID2

D2024.3

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SEQ ID3

F11E6.5

atggcagcagcacaacaagtcacagcagccacgctcgtcgatgttttgacaaaaccatgg
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tacattcaaaaacgctcaccaacccaaaaccagcaagcaggagtag

SEQ ID4

F41H10.7

atgtcatcggacgatcgtggcactagaaccttcaagatgatggatcaaattcttggaaca
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cttgccgctcggatataattgccactatttttggactgaaatattatatgaaagaccgaaaa
gccttcgatctcagtactccattaaacatttggaaatggattctttcgacattcagctta
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gctcaagcaatcaccacatctcaaattgggttcaattcgcagttgccattttcgcacaagtt
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acagctatcggatttttcatgcttactacacttctatctatggattcaattctacaaa
gagcactatcttaagaatggaggcaaaaagtacaatttggcaaaggatcaggcaaaaact
caaacaaagaaggctaactaa

SEQ ID5

F41H10.8 (ce477)

atgccacagg	gagaagtctc	attctttgag	gtgctgacaa	ctgctccatt
cagtcatgag	ctctcaaaaa	agcatattgc	acagactcag	tatgctgctt
tctggatctc	aatggcatat	gttgctcgta	tttttgggct	caaggctgtc
atgacaaacc	gaaaaccatt	tgatctcacg	ggaccactga	atctctggaa
tgccgggtctt	gctattttct	caactctcgg	atcacttgcc	actacatttg
gacttctcca	cgagttcttc	agccgtggat	ttttcgaatc	ttacattcac
atcggagact	tttataatgg	actttctgga	atgttcacat	ggcttttcgt

tctctcaaaa gttgctgaat tcggagatac acttttttatt attcttcgta
aaaagccatt gatgttcctt cattgggtatc atcatgtgct tacaatgaat
tatgctttta tgtcatttga agctaatttg ggatttaata cttggattac
atggatgaat ttctcagttc actcaattat gtatggatat tatatgcttc
gttcttttgg tgtcaagggt ccagcatgga ttgccaagaa tattaacaaca
atgcaaattc ttcaattcgt tattactcat ttcattcttt tccacgttgg
atatttggca gttactggac aatctgttga ctcaactcca ggatattatt
ggttctgctt tctcatggaa atctcttatg tcgttctgtt cggaaacttc
tactatcaat catacatcaa gggaggtggc aagaagtta atgcagagaa
gaagactgaa aagaaaattg aataa

SEQ ID6

F56H11.3

atgtatttgaattatttcgcgacggaaatcttccatcgtagtgcggtttgtgaaacagaa
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ctttga

SEQ ID7

F56H11.4 (Ce 166)

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acgatttgtg gctattgcta ctcatgggcc aaagaatttc cctgacgcaga
aggtcgcaa gttctttgct gatcactttg atgttactat tcaggcttcaa
tcctgtacat
ggtcggttgtg ttccggaacaa aatgggttcatt gcgtaatcgt caaccattcc
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caaaggaatt gtcgactcactgc aaagtgtttg atttcacgaa aggagagaat
ggatactgggt gtggtctct catggcttc aaacttttcg aacttgttga
caccatcttc ttggttctccgtaaacgtcc actcatgttc cttcactggg
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ccaggattca acagatacgg aatttatctt aactttgtcg
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 gccaaactgt gatttcgagc catcagatatt caagctcgca
 gttttcatgg acacaacata cttggtctct ttcgtcaact tcttcctcca
 atcatatggt
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SEQ ID8

Y53F4B.c

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 gcttcggaat ctacgcctca ttctggtgc tattctccag tttcttcaac
 aatgcatatt tggtaaaaaa ggacaagaaa cccgatgtga agaaggatta
 a

SEQ ID9

A

1 MELAEFWNDL NTFTIYGPNH TDMTTYKYKYS YHFPGEQVAD PQYWTILFQK
 51 YWYHSITISV LYFILIKVIQ KFMENRKPFT LKYPLILWNG ALAAFSIIAT
 101 LRFSIDPLRS LYAEGFYKTL CYSCNPTDVA AFWSFAFALS KIVELGDTMF
 151 IILRKRLIF **LHYYHHA**AVL IYTVHSGAEH TAAGRFYILM NYFAHSLMYT
 201 YYTVSAMGYR LPKWVSMTVT TVQTTQMLAG VGITWMVYKV KTEYKLPCQQ
 251 SVANLYLAFV IYVTFAILFI QFFVKAYIIK SSKKSKSVKN **E***

SEQ ID10

B

1 MAKYDYNPKY GLENYSIFLP FETSFDAFRS TTWMQNHWWYQ SITASVVYVA
 51 VIFTGKKVVL IYKSRVITF ESSLQNAIKN RNRKSLNSSQ MFQIMEKYKP
 101 FQLDTPLFVW NSFLAIFSIL GFLRMTPEFV WSWSAEGNSF KYSICHSSYA
 151 QGVTFGWTEQ FAMSKLFELI DTIFIVLRKR PLIFL**HWYHH** VTVMITWHA
 201 YKDHTASGRW FIWMNYGVHA LMYSYYALRS LKFRLLPKQMA MVVTTLQLAQ

251 MVMGVIIGVT VYRIKSSGEY CQQTWDNLGL CFGVYFTYFL LFANFFYHAY
301 VKKNNRTVNY ENNSKNFPDL VLIYLRKKVS RKSKNRQCSE NNYKIQFSSN
351 FVNVDGKKHK KTYELILPRR KMTTILTFLF GKNRIFSKYQ KNRKNISIPV
401 DFEILEPKED INANIAEPSI TTRSAAARRK VQKAD*

SEQ ID11**C**

1 MAAAQTSPAA TLVDVLTKPW SLDQTDSYMS TfvPLSYKIM IGYLVTIYFG
51 QKLMAHRKPF DLQNTLALWN FGFSLFSGIA AYKLIPELFG VFMKDGfVAS
101 YCQENENYYTD ASTGFWGWAF VMSKAPeLGD TMFLVLRKKP VIFMHWYHHA
151 LTFVYAVVTY SEHQAWARWS LALNLAVHTV MYFYFAVRAL NIQTPrpVAK
201 FITTIQIVQF VISCYIFGHL VFIKSADSVp GCAVSWNVLS IGGLMYISYL
251 FLFAKFFYKA YIQKRSPTKT SKQE*

SEQ ID12**D**

1 MSSDDRGRTRT FKMMDQILGT NfTYEGAKEV ARGLEGfSAK LAVGYIATIF
51 GLKYMKDRK AFDLSTPLNI WNGILSTfSL LGFLFTFPTL LSVIRKDGfS
101 HTYSHVSELY TDSTSGYWIF LWVISKIPEL LDTVfIVLRK RPLIFMHWYH
151 HALTGYYALV CYHEDAVHmV WvVWMNYIIH AFMYGYYLLK SLKVPIPPSV
201 AQAITTSQMV QFAVAIFAQV HVSyKHVEG VEGlAYSFRG TAIGFFMLTT
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SEQ ID13**E**

MPQGEVSFFE VLTTAPfSHE LSKKHIAQTQ YAAFWISMAY VVvIFGLKAV
MTNRKPFDLT GPLNLWNAGL AIFSTLGSLa TTFGLLHEFF SRGFFESYIH
IGDFYNGLSG MFTWLFVLSK VAefGDTLFI ILRKKPLMFL HWYHHVLTmN
YAFMSFEANL GFNTWITWMN FSVHSIMYGy YMLRSFGVKV PAWIAKNITT
MQILQfVITH FILFHVGyLA VTGQSVdSTP GYYWfCLLME ISYVVLFGNF
YYQSYIKGGG KKFNAEKkTE KKIE*

SEQ ID14**F**

1 MYLNYFATEI FHRSAVCETE ACRSSKIMIA DVfKWKFDAN ELWSLLTNQD
51 EVFPHIRARR FIQEHfGLFV QMAIAYVILV FSIKRfMRDR EPfQLTTALR
101 LWNFFLSVFS IYGSWTMfPF MVQQIRLYGL YGCGCEALSN LPSQAeyWLF

151 LTILSKAVEF VDTFFLVLRK KPLIFLHWYH HMAFVFFCS NYTPSSQSR
201 VGVIVNLFVH AFMYPYYFTR SMNIKVPAKI SMAVTVLQLT QFMCFIYGCT
251 LMYYSLATNQ ARYPSNTPAT LQCLSYTLHL L*

SEQ ID15**G**

MAQHPLVQRL LDVKFDTKRF VAIATHGPKN FPDAEGRKFF ADHFDVTIQA
SILYMVVVFG TKWFMRNRQP FQLTIPLNW NFILAAFSIA GAVKMTPEFF
GTIANKGIVA SYCKVFDFTK GENGYVWVLF MASKLFELVD TIFLVLRKRP
LMFLHWYHHI LTMIYAWYSH PLTPGFNRYG IYLNFVVHAF MYSYYFLRSM
KIRVPGFIAQ AITSLQIVQF IISCAVLAHL GYLMHFTNAN CDFEPSVFKL
AVFMDTTYLA LFNFFLQSY VLRGGKDKYK AVPKKKNN*

SEQ ID16**H**

MSAEVSEFVKVWTGNNETIIYSPFEYDSTLLIESCRCTYQLLILLRQI
YYRDIWSHGNLACDXLLAWNGFLAVFSIMGTWRFGIEFYDAVFRXG
FIXSICLAVNPRSPSAFWACMFALSKIAEFGDTMFLVLRKRPVIFLHWYHH
AVVLILSWHAAIELTAPGRWFIFMNYLVHSIMYTYAITSIGYRXPKIVSMT
VTFLQTLQMLIGVSISCIVLKLNEMCQQSYDNLALSFGIYASFLVLSSFF
NNAYLVKKDKKPDVKKD*

Claims

1. An isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase as herein defined.
2. A polypeptide according to claim 1 wherein the polypeptide is from a eukaryote.
3. A polypeptide according to claim 1 or claim 2 wherein the polypeptide has at least a portion of the amino acid sequence shown in SEQ ID 15, or variants thereof.
4. A polypeptide having at least 60% homology to a polypeptide according to claim 3 and having a PUFA elongase function.
5. A polypeptide according to claim 4 having at least 80% homology.
6. A polypeptide according to claim 5 having at least 90% homology.
7. A polypeptide according to any preceding claim wherein the polypeptide sequence includes a sequence motif responsible for Endoplasmic Reticulum (ER) - retention.
8. A polypeptide according to any preceding claim wherein the polypeptide is capable of elongating palmitoleic acid (PA; 16:1 Δ^9) to vaccenic acid (VA; 18:1 Δ^{11}).
9. A polypeptide according to any preceding claim wherein the polypeptide is from an animal.
10. A polypeptide according to claim 9 wherein the animal is an invertebrate.
11. A polypeptide according to claim 10 wherein the invertebrate is a worm.
12. A polypeptide according to claim 11 wherein the worm is *C. elegans*.

13. A polypeptide according to claim 9 wherein the animal is a vertebrate.
14. A polypeptide according to claim 13 wherein the vertebrate is a mammal.
15. A polypeptide according to claim 14 wherein the mammal is a human, rat or mouse.
16. A DNA sequence encoding a polypeptide according to any preceding claim.
17. A DNA sequence according to claim 16 wherein the DNA comprises the sequence shown in SEQ ID 7 or variants of that sequence due to base substitutions, deletions and/or additions.
18. An engineered organism engineered to express a polypeptide according to any one of claims 1 to 15.
19. An engineered organism according to claim 18 wherein the animal is a mammal.
20. An engineered organism according to claim 19 wherein the mammal is a rat, mouse or monkey.
21. An engineered organism containing a synthetic pathway for the production of a polypeptide according to any one of claims 1 to 15.
22. An engineered organism according to claim 21 wherein the pathway includes Δ^5 -fatty acid desaturase.
23. An engineered organism according to claim 21 or 22 wherein the pathway includes Δ^6 -fatty acid desaturase.
24. An engineered organism according to any one of claims 21 to 23 wherein the animal is a lower eukaryote.

25. An engineered organism according to claim 24 wherein the lower eukaryote is a yeast.
26. An engineered organism according to claim 18 wherein the animal is a fish.
27. A transgenic plant engineered to express a polypeptide according to any one of claims 1 to 15.
28. A transgenic plant containing a DNA sequence according to claim 16 or 17.
29. A method of producing a PUFA comprising carrying out an elongase reaction catalysed by a polypeptide according to any one of claims 1 to 15.
30. A method according to claim 29 wherein the PUFA is di-homo-gamma-linoleic acid ($20:3\Delta^{8,11,14}$), arachidonic acid ($20:4\Delta^{5,8,11,14}$), eicosapentanoic acid ($20:5\Delta^{5,8,11,14,17}$), docosatrienoic acid ($22:3\Delta^{3,16,19}$), docosatetraenoic acid ($22:4\Delta^{7,10,13,16}$), docosapentaenoic acid ($22:5\Delta^{7,10,13,16,19}$) or docosahexaenoic acid ($22:6\Delta^{4,7,10,13,16,19}$).
31. A method according to claim 29 wherein the PUFA is a 24 carbon fatty acid with at least 4 double bonds.
32. A PUFA produced by a method according to any one of claims 29 to 31.
33. A foodstuff comprising a PUFA according to claim 32.
34. A dietary supplement comprising a PUFA according to claim 32.
35. A pharmaceutical composition comprising a polypeptide according to any one of claims 1 to 15.
36. A pharmaceutical composition comprising a PUFA according to claim 32.

37. A pharmaceutical composition according to claim 35 or claim 36 wherein the composition comprises a pharmaceutically-acceptable diluent, carrier, excipient or extender.
38. A method of elevating the PUFA levels of an animal or a plant by supplying to the animal or plant a polypeptide according to any of claims 1 to 15, a DNA sequence according to claim 16 or claim 17, a foodstuff according to claim 33, a dietary supplement according to claim 34, a pharmaceutical composition according to any of claims 35 to 37 or a PUFA according to claim 32.
39. A method of treatment according to claim 38 wherein the animal is a mammal.
40. A method of treatment according to claim 39 wherein the mammal is a human.

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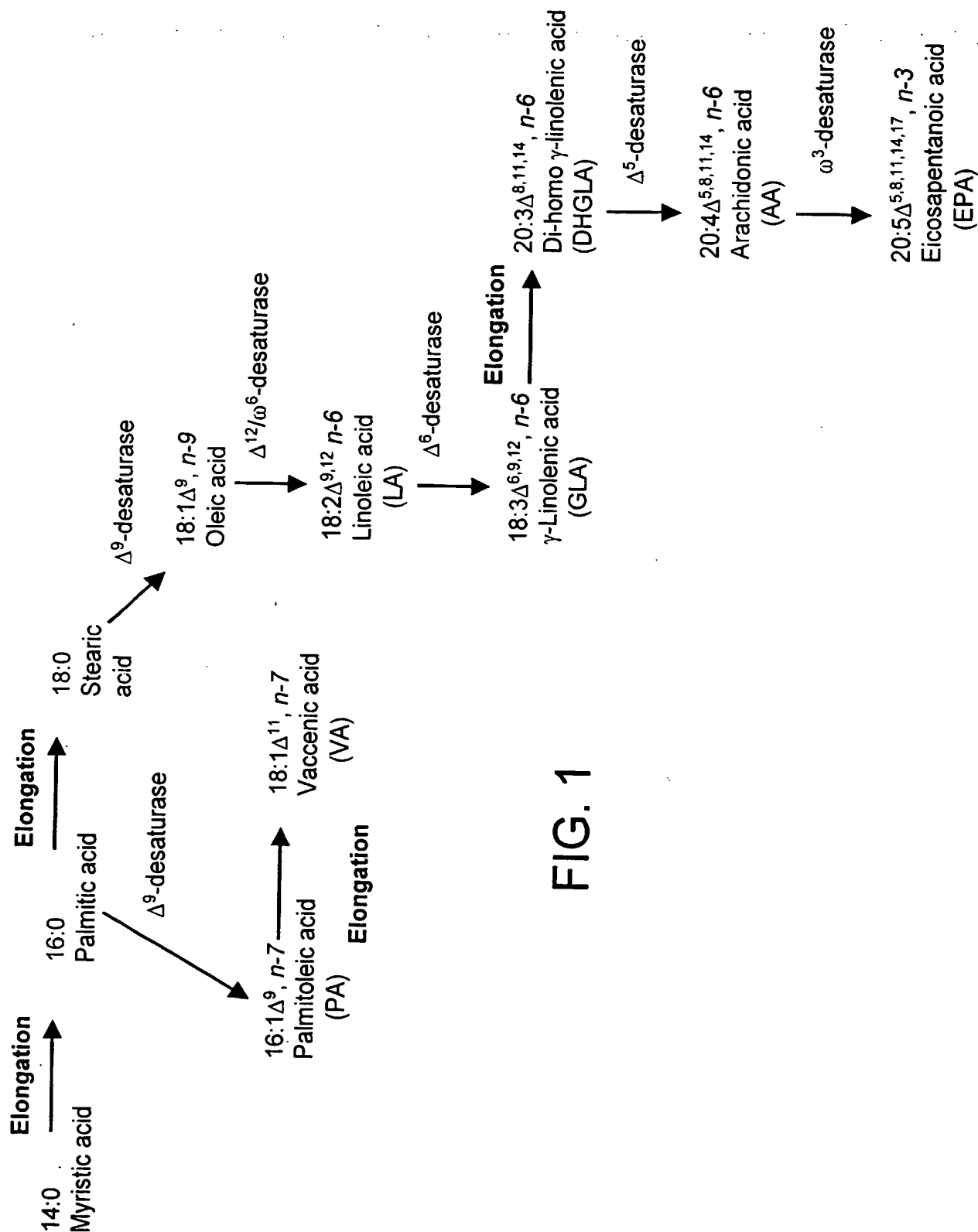


FIG. 1

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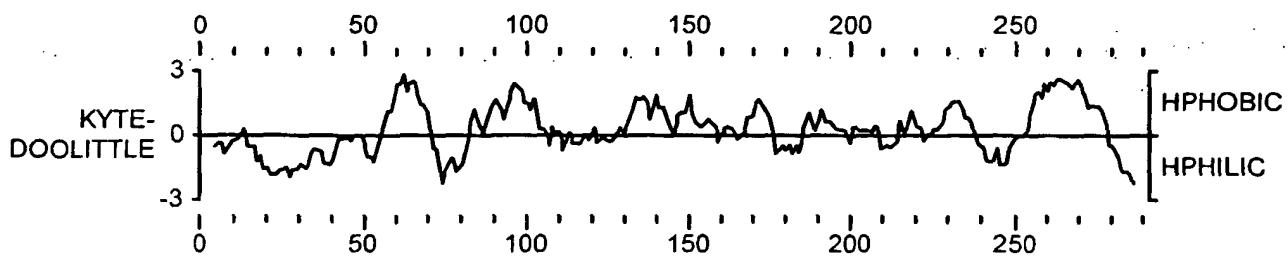


FIG. 2

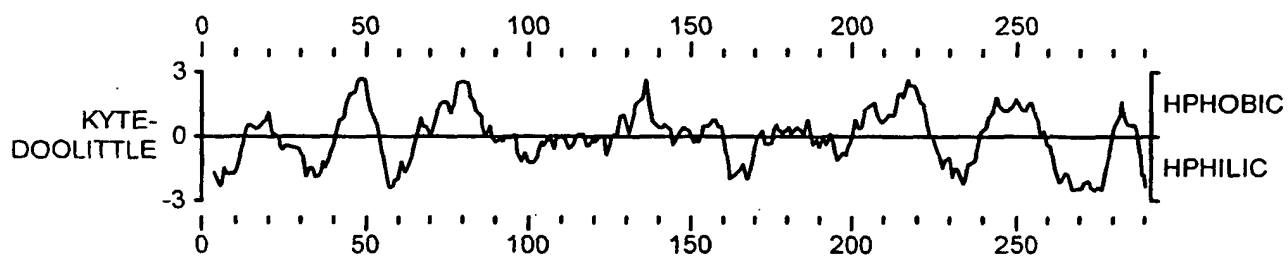


FIG. 3

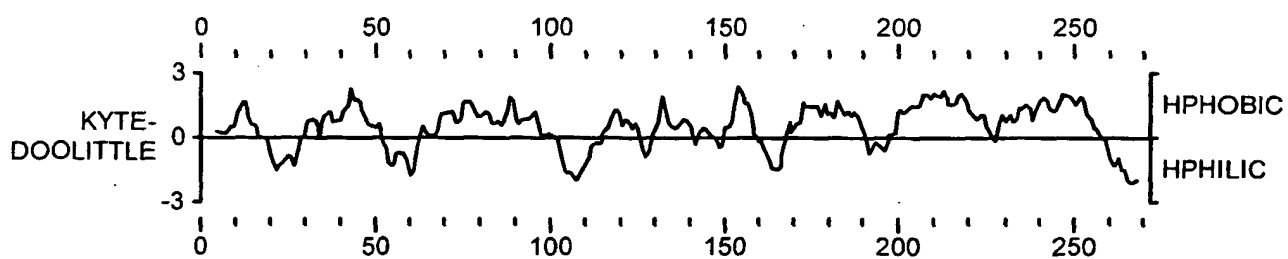


FIG. 4

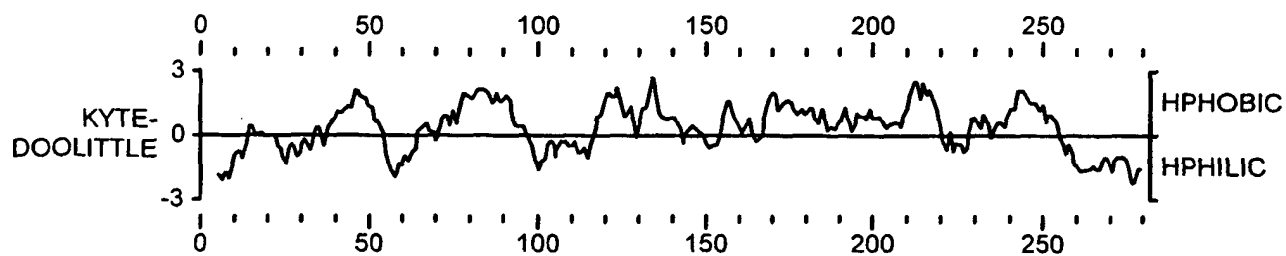


FIG. 5

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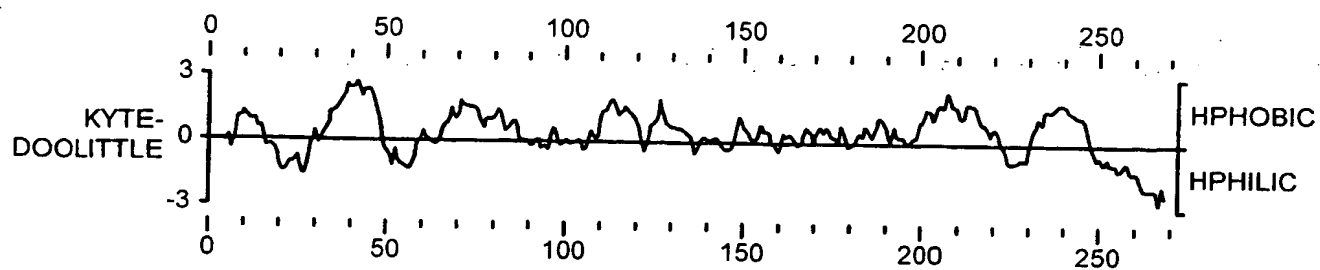


FIG. 6

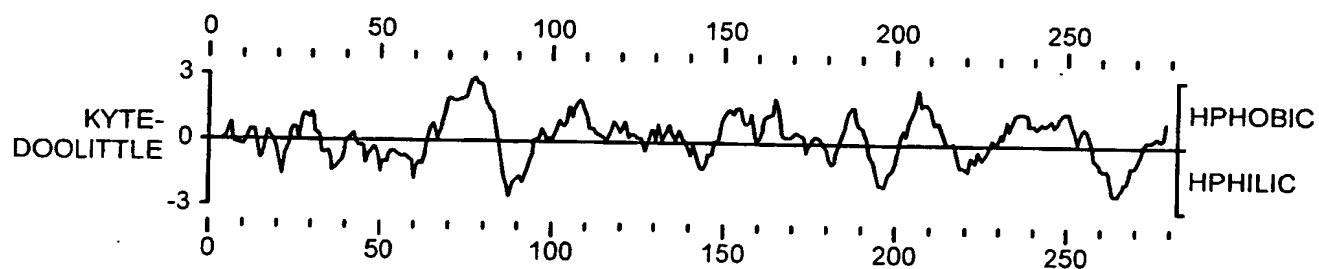


FIG. 7

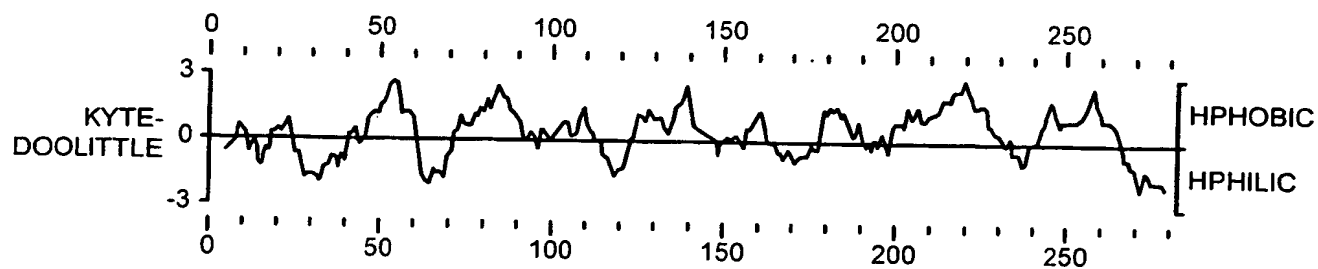


FIG. 8

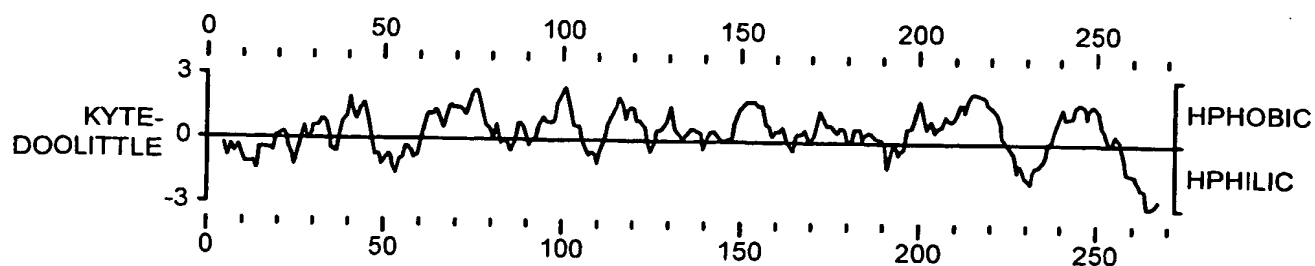


FIG. 9

Elo1	V L R K K P L I F F L H W Y H H M A T F V F C S N Y P T P S E A N L G F N T W I T W N F S V H A F M Y P P Y Y F T R S M N I R V
Elo2	V L R K K P L I F F L H W Y H H M A T F V F C S N Y P T P S E A N L G F N T W I T W N F S V H A F M Y P P Y Y F T R S M N I R V
Elo3	V L R K K P L I F F L H W Y H H M A T F V F C S N Y P T P S E A N L G F N T W I T W N F S V H A F M Y P P Y Y F T R S M N I R V
Cig30	V L R K K P L I F F L H W Y H H M A T F V F C S N Y P T P S E A N L G F N T W I T W N F S V H A F M Y P P Y Y F T R S M N I R V
B207d4	V L R K K P L I F F L H W Y H H M A T F V F C S N Y P T P S E A N L G F N T W I T W N F S V H A F M Y P P Y Y F T R S M N I R V
F56H11.4	V L R K K P L I F F L H W Y H H M A T F V F C S N Y P T P S E A N L G F N T W I T W N F S V H A F M Y P P Y Y F T R S M N I R V
F4H10.8	V L R K K P L I F F L H W Y H H M A T F V F C S N Y P T P S E A N L G F N T W I T W N F S V H A F M Y P P Y Y F T R S M N I R V
F56H11.3	V L R K K P L I F F L H W Y H H M A T F V F C S N Y P T P S E A N L G F N T W I T W N F S V H A F M Y P P Y Y F T R S M N I R V

[illegible]

Elo1	L T S Y L F	L F	S F Y I E V Y K R	G S A	S G K K K	I N K N N	- - - - -	- - - - -
Elo2	I S Y L V	L F S F Y I V V Y K R	K G T K T S R V V K R A H G G V A A K V N E Y V N V D L K N V P T P S P K P Q H	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
Elo3	L T S Y L	L F S F Y I O S Y K R	G K K T V K K E S V S G S V A S G S T G V K T S N T K V S S R K A	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
C1930	Y G T Y F	L F A H F F H R A Y I R	P K C K V A S K S Q	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
B207d4	Y L S Y L	L F C H F F E A Y I	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
F56H11.4	D T Y Y L A	L F V N F F O S Y I R	G C K D K Y K A V P K K K N N	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
F4H10.8	E I S Y V V	L F G N F Y I O S Y I R	G G K - K F N A E K K T E K K I E	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
F56H11.3	Y T L H L	L	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -

Elo1	-	-	-
Elo2	R	R	K
Elo3	-	-	-
Cig30	-	-	-
B207d4	-	-	-
F56H11.4	-	-	-
F41H10.8	-	-	-
F56H11.3	-	-	-

FIG. 10 CONT'D

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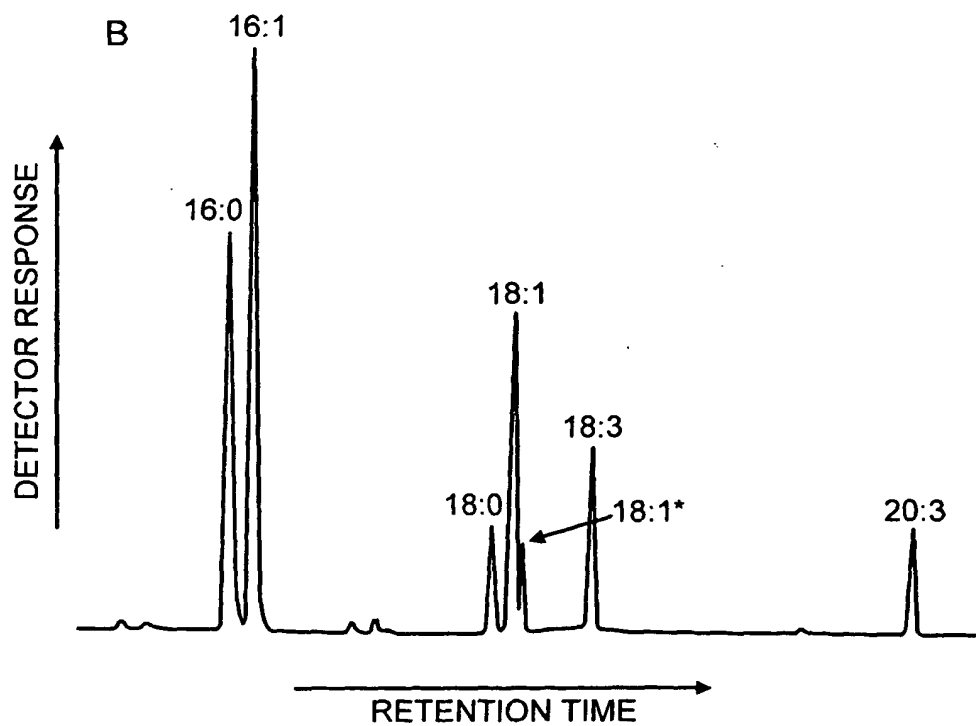
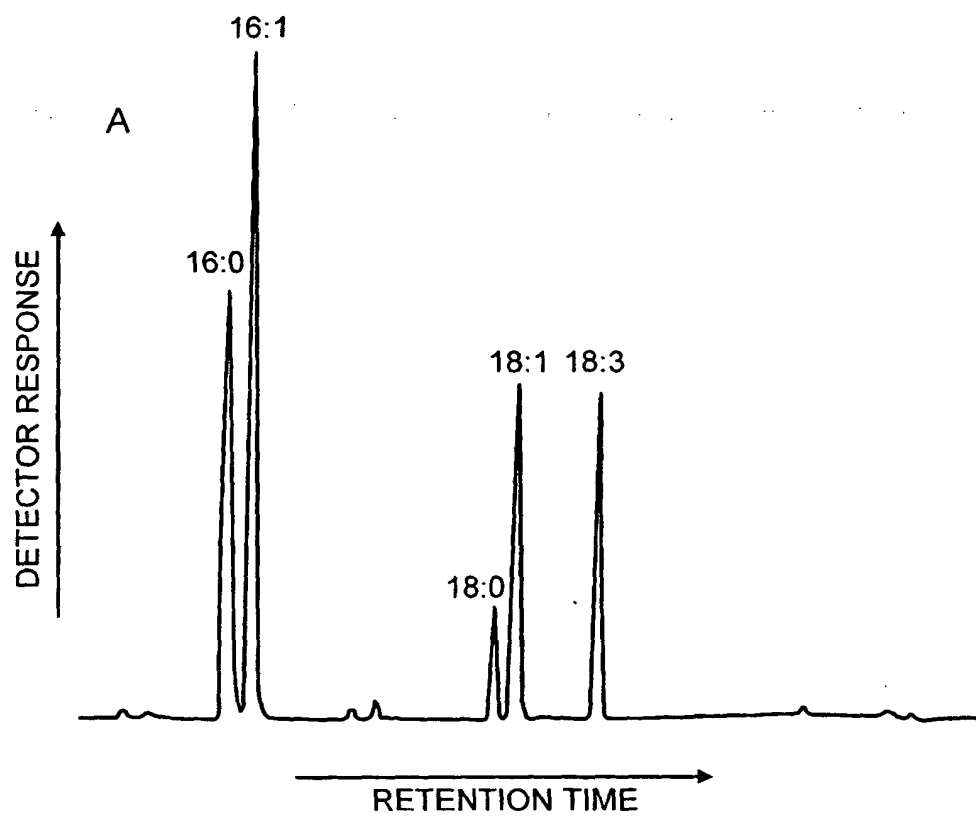


FIG. 11

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01035

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/54 C12N9/10 C12N1/21 A01H5/00 C07C57/03
 C07C57/12 A61K38/45 A61K31/202 A23L1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE SWALL 'Online! EMBL Heidelberg, Germany; ID: YLF4_CAEEL, AC: Q03574, 1 February 1994 (1994-02-01) WILSON R ET AL.: "2.2 Mb of contiguous nucleotide sequence from chromosome III of C. elegans" XP002143744 abstract</p> <p style="text-align: center;">--- -/-</p>	1-12, 18, 21, 24



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

28 July 2000

Date of mailing of the international search report

17. 08. 00

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Authorized officer

Oderwald, H

INTERNATIONAL SEARCH REPORT

Int lional Application No

PCT/GB 00/01035

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMINV 'Online! EMBL Heidelberg, Germany; ID: CEC40H1, AC: Z19154, 27 December 1992 (1992-12-27) BERKS M: "Caenorhabditis elegans cosmid C40H1" XP002143745 see nucleotides 18500 to 20600 abstract</p> <p>---</p>	16,17
X	<p>JAMES D W ET AL: "DIRECTED TAGGING OF THE ARABIDOPSIS FATTY ACID ELONGATIONI (FAE1) GENE WITH THE MAIZE TRANSPOREN ACTIVATOR" PLANT CELL,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 7, March 1995 (1995-03), pages 309-319, XP002911493 ISSN: 1040-4651 cited in the application the whole document</p> <p>---</p>	1-3,7,8, 16-18, 21-23, 27-29
A	<p>WATTS J L AND BROWNE J: "Isolation and characterization of a delta5-fatty acid desaturase from Caenorhabditis elegans" ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 362, no. 1, 1 February 1999 (1999-02-01), pages 175-182, XP002143742 the whole document</p> <p>---</p>	22
A	<p>NAPIER ET AL: "Identification of a Caenorhabditis elegans delta6-fatty-acid-desaturase by heterologous expression in Saccharomyces cerevisiae" BIOCHEMICAL JOURNAL,GB,PORTLAND PRESS, LONDON, vol. 330, no. 2, March 1998 (1998-03), pages 611-614-614, XP002099453 ISSN: 0264-6021 the whole document</p> <p>---</p>	23
A	<p>SALEM N ET AL: "Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 93, no. 93, January 1996 (1996-01), pages 49-54-54, XP002131822 ISSN: 0027-8424 the whole document</p> <p>---</p>	

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01035

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OH ET AL: "ELO2 and ELO3, homologs of the Saccharomyces cerevisiae ELO1 gene, function in fatty acid elongation and are required for sphingolipid formation" JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 28, 11 July 1997 (1997-07-11), pages 17376-17384, XP002119019 ISSN: 0021-9258 cited in the application the whole document	
P, X	WO 00 12720 A (ABBOTT LAB) 9 March 2000 (2000-03-09) the whole document	1-3, 7-31, 35

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/01035

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 38-40 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Information on patent family members

PCT/GB 00/01035

Form PCT/ISA/210 (patent family annex) (July 1992)